MYCOLOGIA

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SOME LEAFSPOT FUNGI ON WESTERN GRAMINEAE–XIII[1]

RODERICK SPRAGUE[2]

(WITH 2 FIGURES)

The material collected by G. W. Fischer and the writer in the Southwest in late summer of 1957 has been discussed in part in the last paper in this series (17), but there proved to be too much noteworthy material in the 1957 collections to include all of it one issue of a periodical.

Besides reports of noteworthy specimens collected by my colleagues and myself I have included a few specimens obtained from mounted sheets of grasses filed in the Department of Botany Herbarium, Washington State University, Pullman. Earlier I reported on about 130 specimens removed from grasses arranged alphabetically partly through the genus *Bromus* (**15**). The additions from this source include only through *Bromus sitchensis* Trin. The bulk of the phanerogamic specimen sheets remain unexamined. Because available time has not permitted any substantial study of this backlog, the writer prefers to include the small amount of additional data in the body of the current paper.

Scirrhia pluristriae sp. nov.

In foliis stramineis, stromatibus linearibus, erumpentibus, inter nervos paralleliter dispositis, raro confluentibus, nigris, 200–470 × 50–85 μ, 1–6 loculos paralleliter

[1] Scientific Paper No. 1837. Washington Agricultural Experiment Stations, Pullman, Washington. Project No. 449.

[2] Pathologist, Washington State University, Tree Fruit Experiment Station, Wenatchee, Washington.

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dispositos, 35–60 μ diam, albofarctos concludentibus; ascis clavatis, aparaphysis 35–43 × 12–14 μ; sporulis chlorinaceis-hyalinis, subreniformibus v. fusoideis, 14–20 × 4.0–5.8 μ.

Growing in straw-colored leaves, stroma linear, pointed at either end, erumpent to subsuperficial, emerging by splitting the leaf epidermis, somewhat vein-delimited, only occasionally confluent, black in mass, the mycelium creosote brown, 200–470 × 50–85 μ diam; containing 1 to 6, most often 3 loculi which contain hyaline or chlorine material which gives them a lighter appearance than the remainder of the fruiting body, the apex thinner, gradually breaking to permit emergence of asci when moistened, the loculi averaging 35–60 μ, globose; asci hyaline, club-shaped, without paraphyses, 35–43 × 12–14 μ, capable of some elongation in water mounts; spores 8 per ascus, subreniform to fusoid, yellow-hyaline, 14–20 × 4.0–5.8 μ.

Hab. in lower yellow leaves of living plants of *Muhlenbergia asperifolia* (Nees and Mey.) Par., three miles south of Sprague, Lincoln County, Washington, September 4, 1956. TYPE is WSP 42854.

The absence of paraphyses excludes this fungus from *Placostroma*. I compared this, however, with *P. bambusae* (Turc.) Sprague and with *P. sporoboli* (Atk.) Sprague (**13**). In addition to the presence of paraphyses in the latter two they also have appreciably larger stromata, locules and asci than *S. pluristriae*. The latter has much smaller spores than *P. bambusae* and slightly larger ones than *P. sporoboli*. Possibly the superficial similarity of the three species has permitted *S. pluristriae* to escape earlier notice. The latter is abundant on the lower half of the foliage of the grass. It may be mildly parasitic.

Bipolaris arizonica sp. nov.

Maculis diffusis, emarginatis, siccis, griseis v. sordide stramineis, conidiophoris diffusis v. subgregariis, brunneis, 4–9-septatis, subgeniculatis, 57–85 × 6–7.2 μ; conidiis fusiformicylindraceis, brunneis, 3–5(–6)-septatis, 33–56 × 8.3–12.0 μ.

Spots diffuse, emarginate, dry, gray or pale gray to dirty straw color; conidiophores scattered widely over the diseased areas, numerous, often single or sometimes in pairs, brown from brown mycelia, somewhat geniculated toward the apex with the apical areas subhyaline, 4–9-septate, 57–85 × 6.0–7.2 μ; conidia fusiform-cylindrical, widest usually between the second and third septum in longer spores, brown, 3–5–(–6)-septate, hilum enclosed in the cell wall, 33–56 × 8.3–12.0 μ, spores germinating from distal cells.

Hab. in living plants of *Muhlenbergia wrightii* Vasey, along the Snow Bowl Road out of Flagstaff, Arizona, September 3, 1957. TYPE is WSP 46123.

This fungus produces a firm to slightly cobwebby nearly black growth in pure culture. The spores germinate from distal cells (FIG. 1, E).

I have encountered *Helminthosporium* spp. from time-to-time that I suspected were undescribed but never had the temerity to name one because of the exceptional research done by earlier workers, notably Drechsler (**5**), on the species of Gramineae. In recent years the writer has not had very great luck in obtaining aid on the determination of these western unknowns.[3] Since I cannot find any fungus to which I can refer the *Muhlenbergia* material, I have decided to describe it. I have not been able to find any mention of a *Helminthosporium* on *Muhlenbergia* from the western United States except an undescribed leaf blotch which I once collected on *M. asperifolia* from Ft. Totten, North Dakota, and some soil-borne forms including *H. halodes* Drechsl. and *H. sativum* P.K.B. (= *B. sorokiniana* (Sacc. in Sorok.) Shoemaker) on *M. racemosa* (Michx.) B.S.P. from Gardner, North Dakota (**18**).

BIPOLARIS BRIZAE (Nisikado) Shoemaker was collected on ripened plants *Gastridium ventricosum* (Guoan) Schenz and Thell. near Copperopolis, Calif., Aug. 29, 1957 (WSP 46126). Before seeing Shoemaker's paper [4] I described this, in manuscript, as *Helminthosporium gastridii*. I submitted material to Shoemaker who believes that this fungus is identical with *B. brizae*. I am withdrawing the formal description of *B.* (*H.*) *gastridii*, but since a description was made in some detail, I am submitting it for comparison:

Spots diffuse, rarely with remnants of a margin, spread over yellowed to pale tawny dried leaves; mycelium in tissue prominent, tortuous or growing in long strands between veins, septate, coarse, mostly about 7 μ diam, dark red-brown; conidiophores arising from a basal pad about 10 μ diam, single, less often in pairs, rarely branched, 3–7–16-septate, somewhat geniculate near apex, 30–100 × 7 μ, producing spores from apex sometimes in threes; conidia dark brown, fusiform-cylindric, hilum prominent, hilum cell sometimes lighter colored than remainder of spore, 2–5-septate, 40–56 × 10.3–11 μ, some immature 1–2 celled spores are smaller.

[3] Shoemaker, R. A. Nomenclature of *Drechslera* and *Bipolaris*, grass parasites segregated from *Helminthosporium*. Canad. J. Bot. **37**: 879–887. 1959. This reference appeared after my paper was submitted and approved for publication on April 24, 1959. At this date, Feb. 10, 1961, the original manuscript has not been located nor transmitted to the present editor although reported once in 1959 to be in galley. The delay has at least given me time to include Shoemaker's determinations of some of the so-called *Helminthosporium* spp.

[4] Loc. cit.

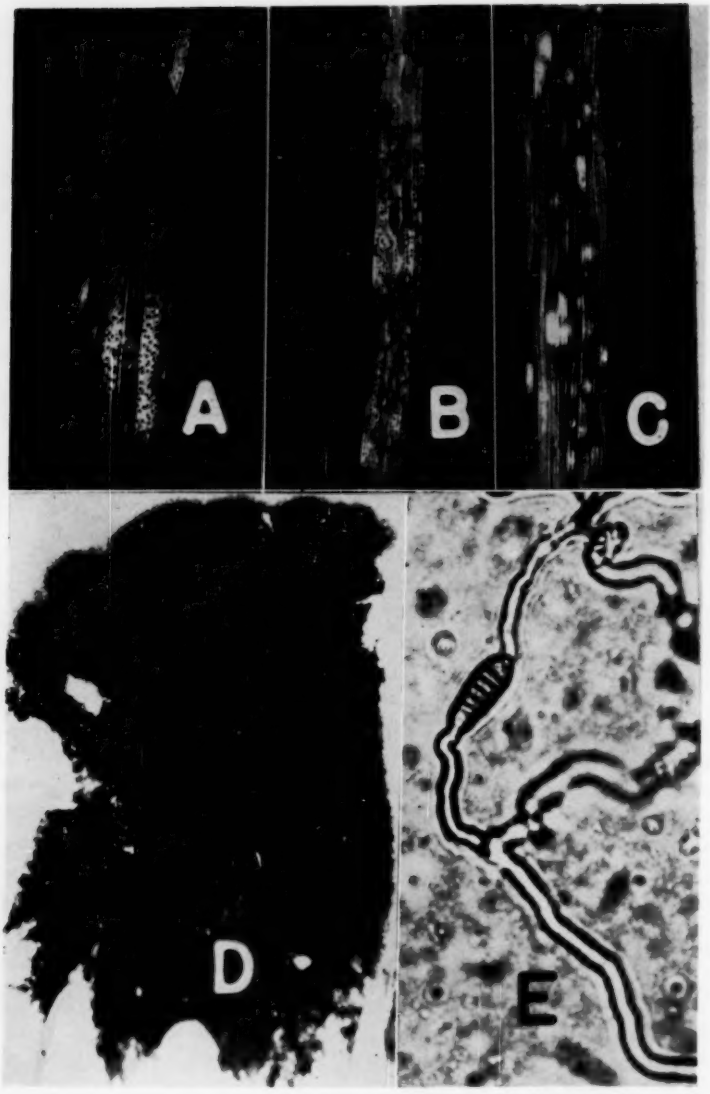

FIG. 1, A-E.

FIG. 1, A. *Septoria calamagrostidis* f. *koeleriae* on *Koeleria phleoides*. Inst. Speg. 21835, × 3. FIG. 1, B. *Mycosphaerella maydis* on *Digitaria decumbens*, Panama. WSP 46197, × 2. FIG. 1, C. *Ovularia pusilla* on *Agrostis alba*, Blicken-

The fungus produces a slightly cobwebby, nearly black growth on PDA (FIG. 1, D) which is similar in color to *B. arizonica*, but tends to form some cobwebby or stringy strands and with less tendency to form a staling overgrowth of mouse-gray aerial mycelium.

The only other report of *Bipolaris* on nit grass is one from the southern Willamette Valley found on a phanerogamic specimen at Oregon State College about 20 years ago (**13**). That fragment consisted of one sharply defined white spot with a narrow vinaceous border. The spores were dark, similar to the Copperopolis material. There are only faint indications of a defined spot in the "type," however, which is dead, dried summer material found on the parched foothills of California.

DRECHSLERA TRITICI-REPENTIS (Died.) Shoemaker caused a dark brown blotch on living leaves of *Agropyron subsecundum* (Lk.) Hitchc. in the Geaudreau Meadows 4 miles north of Blanchard, Idaho (WSP 46115). This fungus had been found previously on this host in Alaska and North Dakota.

DRECHSLERA BROMI (Died.) Shoemaker was the cause of a common blight on leaves of *Bromus vulgaris* (Hook.) Shear near Chewack Creek, Okanogan County, Washington (WSP 46096) in 1957 and along the Entiat River Trail in Chelan County, Washington (WSP 40216) on September 14, 1958.

DRECHSLERA AVENACEA (Curt. ex Cke.) Shoemaker occurs with a leaf tip blight of *Trisetum spicatum* (L.) Richt. at the start of the trail on Bald Mountain, Wasatch National Forest, Utah (WSP 46032). A few yellowish to red-bordered spots were also found on the main body of the leaves as well as on the yellowed tips. The yellowish, thin-walled, slightly tapered conidia borne on short stout yellowish conidiophores are typical of *D. avenacea*. Spores seen measured 49–78 × 13–15 μ. I can find no report of *Drechslera* (*Helminthosporium*) on the genus *Trisetum* from our records, nor in Saccardo (**7**), nor Seymour (**9**). The collection area lies at the base of an alpine peak in a small grassy, rocky meadow. The place is well known for its frequent, sudden, violent summer storms. Associated were unusual material reported elsewhere in this paper and in the recent paper of this series (**17**). It is indicated that the frequent and fierce mountain storms favored the development of fungi on otherwise highly resistant hosts during the growing season.

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derfer Creek, Washington (WSP 46067), × 3. FIG. 1, D. Pure culture of *Bipolaris brizae* (Culture 5040). (Removed from culture tube for photographing), × 4. FIG. 1, E. Germinating spore of *B. arizonica* (Culture 5041), × 290. (Photo. 1, A–E, by Peggybeth Figaro).

Physoderma alpina sp. nov.

Maculis ellipticis, saepe confluentibus, tarde erumpentibus, brunneis v. fuligineis, 300–400(–600) μ longis; rhizomycelio tenui, sporulis perdurantibus endobioticis, cellulis mesophyllum implentibus, aureobrunneis, globosis v. ovato-ellipsoideis, 26–30 $\times$ 19–26 μ.

Spots elliptical or fusiform elliptical becoming confluent, finally erumpent or eroding to expose a powdery mass, brown to fuliginous, 300–400(–600) μ long; rhizomycelium tenuis; resting spores endobiotic, filling the mesophyll cells in elongated areas, golden brown, globose to ovate ellipsoid, 26–30 $\times$ 19–26 μ.

In living leaves of *Poa gracillima* Vasey at the start of the trail up Bald Mountain, Wasatch National Forest about 30 miles east of Kamas, Utah, September 5, 1957. TYPE is WSP 46026.

This fungus, on a new host for *Physoderma* in western America, is close to *P. maydis* Miyabe, especially in spore size which is virtually identical. The material on *Poa* has possibly brighter spores than the species on *Zea mays* L. The pustules on *Poa* seem to be slower to break open than those on *Zea*. The extreme difference in environment and type of host tends to suggest that the Utah form is not related to the corn disease organism. On the basis of morphology, however, the form is apparently related to the well-known *P. maydis*. While I first called this a form of *P. maydis*, in final analysis it seems impractical to include this obviously different, if morphologically similar fungus, with the warm-temperature-loving *P. maydis*. It also has some resemblance to a fungus called *Entyloma spragueanum* Zundel which also needs further study.

After completing my study of the type of *P. alpina* I encountered the same fungus on *Poa reflexa* Vasey at Mirror Lake, about 5 miles further east from the type area (WSP 46033). The pustules in the material on *P. reflexa* were circular.

In some spore mounts made from the above type I encountered spores of *Spermospora subulata* (Sprague) Sprague, or facsimiles of them, growing on short, hyaline conidiophores in small groups. It was not clear whether these spores were growing on the surface of unbroken pustules of the *Physoderma* or adjacent to them. The pustules and adjacent tissue in such cases were fuliginous. The symptoms in this material were further confused by insect leaf-miners and associated pests in part of it. The culm leaves bearing the complex of fungi were stunted and sometimes recurved, nearly dead, to the base of the leaf. In some leaves I also noted a *Heterosporium* with short stocky yellow-

brown conidiophores bearing young yellowish ovate spores which become brown, septate dolioform spores which were not referable to the common *H. avenae* Oud. One dry glass slide mount of this is filed as WSP 46027 labelled *Heterosporium* sp.

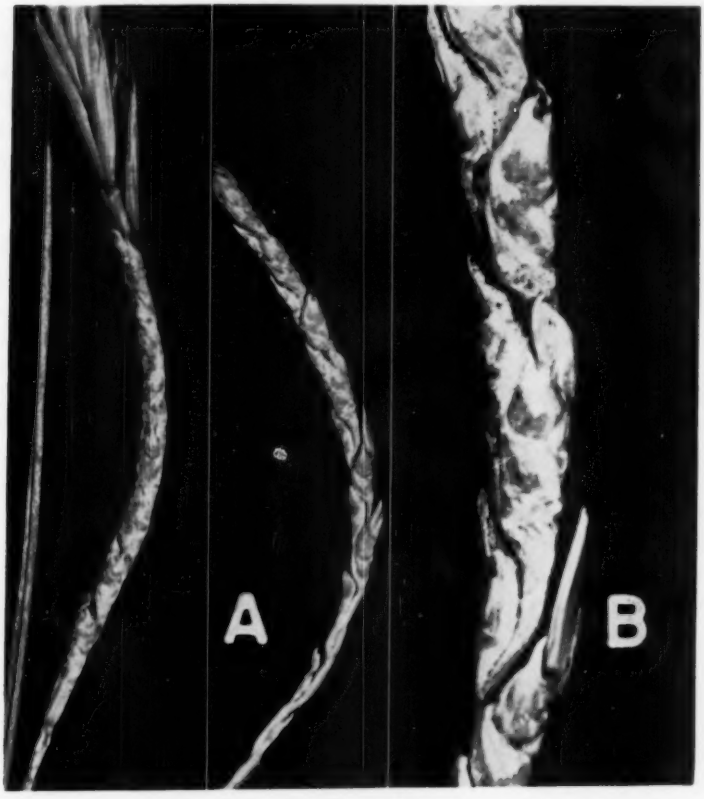

FIG. 2, A. *Epichloe typhina* on heads of *Agropyron trachycaulum*, Pullman, Washington, ×2. (Photo. by Peggybeth Figaro). FIG. 2, B. Same as 2, A, enlarged further, ×7. (Photo. by P. Figaro and Elliott Degman.)

PHYSODERMA BECKMANNIAE Sprague was found in trace amounts on necrotic leaves of *Beckmannia syzigachne* (Steud.) Fern. at Nicola, B. C. (WSP 46247). *"Helminthosporium"* sp. was also present. The last mentioned fungus is common on slough grass in western regions. It may be undescribed (cf. **13**).

CLAVICEPS PURPUREA (Fr.) Tul. produced a few sclerotia in heads of *Lolium subulatum* Vis. near Livermore, California (WSP 46133). Moldy leaves of this collection showed urediniospores of *Puccinia coronata* Corda parasitized by *Clasterosporium* sp.

Claviceps purpurea occurs in the conidial or honey-dew stage on ripening seed of *Festuca ovina* L. in the Geaudreau Meadows 4 miles north of Blanchard, Idaho (WSP 46068). The fungus is unreported in our list (**18**) on sheep's fescue probably because this fescue, like some others, ripens its seed too early for sclerotia to form. While the fescues are very susceptible to ergot and often suffer heavy reduction in seed yield, they are, in some areas and on some species, seldom attacked early enough to permit sclerotia to form.

PHLEOSPORA IDAHOENSIS Sprague caused a black-stem disease of *Festuca ovina* associated with the above-mentioned Idaho ergot. Most of the lesions were sterile or contained a few microspores. This sterile phase is common in northern Idaho in June and July during some years. I found it not only in 1957, but the next year at the same place in early July. I also found *P. idahoensis* on *F. occidentalis* Hook. in dry woods some miles away near Blickenderfer Creek, Pend Oreille County, Washington (WSP 46059). This material had a few macrospores faintly 1–3-septate and as much as 62 μ long. Microspores were present also. Some better material on *F. ovina* with considerable amounts of microspores was studied later near Blanchard, Idaho (WSP 46069; 46078).

DILOPHOSPORA ALOPECURI (Fr.) Fr. was found on a new host, *Hesperochloa kingii* (S. Wats.) Rydb., about 1 mile from Monte Cristo Camp near Monte Cristo Peak, Utah, September 6, 1957 (WSP 46017). Associated also were some prosenchymatous-filled globose fruiting bodies. Not far away on Mt. McKinnon, Utah, I found more of these bodies (WSP 46035). From evidence obtained at various times I question if these are related to the *Dilophospora*. These are more frequently associated with *Selenophoma everhartii* (Sacc. and Syd.) Sprague and A. G. Johnson. *D. alopecuri* was collected on *Stipa lettermani* Vasey at the Iron County line on State Highway 14, Utah, September 4, 1957 (WSP 46045). The small brown lesions on the slender leaves were not conspicuous, not at all as striking as the symptoms of this fungus on *Sitanion, Holcus* and some other host-genera. The genus *Stipa* has not been reported as a host for this fungus in the West.

SELENOPHOMA EVERHARTII produces small, very obscure pycnidia on the heads of *Festuca scabrella* Torr. 10 miles north of Kiowa, Montana (WSP 46119). Wine red immature eyespots were also scattered on the drying culms and leaves. Noted in passing was a small amount of

the uncommon rust *Puccinia cockerelliana* Bethel on *F. scabrella* (WSP 46120), apparently not listed from Montana. I also noted a fungus similar to *Leptostromella* (WSP 46121) which was of questionable parasitic nature.

S. everhartii occurs on pedicels on the dried heads of *Deschampsia caespitosa* (L.) Beauv. in a swamp 7 miles south of Huntsville, Utah (WSP 46013). The spores are arcuate and resemble *S. everhartii*, but are large, 14–21 × 2.0–2.7 μ. There were a few spores of *Scolecotrichum graminis* Fckl. associated with this fungus on the leaves, as well as a scattering of *Septoria poliomela* Sydow (WSP 46014). *Sel. everhartii* occurs on dried leaves of *Calamagrostis inexpansa* A. Gray near Aspen Grove, B. C. (WSP 46250).

SELENOPHOMA DONACIS (Pass.) Sprague and A. G. Johnson is abundant on the pedicels and dried parts of *Poa canbyi* (Scribn.) Piper 15 miles north of Lapine, Oregon (WSP 46005). It is unreported on this host from Oregon but is no doubt common. The fungus was found on the culms of *Agropyron repens* (L.) Beauv. at Sorrento and at Stump Lake, B. C. (WSP 46219), and on *A. trachycaulum* (Lk.) Malte at McKenzie Lake near Princeton, B. C. (WSP 46222) and at Oyama, B. C. (WSP 46235) on September 18, 1958. The fungus was also present on *Elymus cinereus* Scribn. and Merr. at Shuswap, B. C. (WSP 46223), and on culms of *Poa ampla* Merr. at Stump Lake (Hwy. 5), B. C. (WSP 46226). A trace was found on the hybrid *Elymus macounii* Vasey near Nicola, B. C. (WSP 46275).

SELENOPHOMA DONACIS var. STOMATICOLA (Bauml.) Sprague and A. G. Johnson was present on dried culms of *Hordeum jubatum* L. at Knutsford, B. C. (WSP 46225). What we must call the same fungus occurs as a stem speckle on *Deschampsia elongata* (Hook.) Munro near Kemp, B. C. (WSP 46228). The spores range beyond the length of *S. everhartii* and are less arcuate. In our original study of the genus of *Selenophoma* we assigned most of the material on *Deschampsia* to *S. donacis* var. *stomaticola* (**19**). Later we reconsidered this group, partly on the basis of better materials, and placed all but one collection in *S. everhartii* (**18**). In another case I omitted reference to this one collection (**13**, p. 207) but did refer to it as assigned to group 9 (**13**, p. 208). The stem speckle from Canada is comparable to a collection from Mt. Rainier which was assigned to var. *stomaticola* (**18**). This last mentioned fungus, incidentally, was very common in British Columbia in 1958. It was found on dried material of *Koeleria cristata* (L.) Pers. at Knutsford (WSP 46232). It was present on *Poa palustris* L. near Sorrento (WSP 46238) causing a stem speckling. The

spores were small and the pycnidia very obscure. The same fungus caused a severe blight of *Poa pratensis* L. in open woods near Aspen Grove, B. C. (WSP 46248). Associated but in separate lesions was *Erysiphe graminis* DC. ex Merát and *Puccinia poae-sudeticae* (West.) Jørstad.

SELENOPHOMA OBTUSA Sprague and A. G. Johnson was found on leaf tips of *Agropyron spicatum* (Pursh) Scribn. and Sm. along Rim Drive, Crater Lake, Oregon by Dorothy A. Shaw, July 8, 1957 (WSP 46025). The large prominent pycnidia in light spots were typical. The spores varied from the common aberrant blunt condition to some that equalled *S. donacis* in outline. *S. obtusa* is not as common on *A. spicatum* as on *Elymus* and *Sitanion* and this report is new to Oregon. It was found on *Agropyron trachycaulum* on Mt. McKinnon, Utah (WSP 46117), and on *Sitanion hystrix* (Nutt.) J. G. Sm. at Crater Lake, Oregon (WSP 46142).

SELENOPHOMA BROMIGENA (Sacc.) Sprague and A. G. Johnson was found on heads and dried leaves of *Bromus inermis* Leyss at Nicola, B. C. (WSP 46249). No evident lesions were found in this dry material.

MACROPHOMA SPOROBOLI Sprague which is not new to Arizona (**12**) was found on another host genus, *Bouteloua*, on the annual grass *B. barbata* Lag. at Kingman, Arizona (WSP 46122). The fungus is almost identical to the type on *Sporobolus giganteus* Nash from Winslow, Arizona (WSP 3609). The spores in the Kingman material vary from short cylindric to almost subglobose, 16–19 × 6.5–9.0 μ and have the milk-white opaque contents of *Macrophoma* spores. The black pycnidia, 70–140 μ, are scattered in dead, dried, white basal leaves of otherwise vigorous green plants. The area had been favored by a local rain of considerable duration. The fungus is the first sphaeropsidaceous fungus seen on six-weeks grama.

SEPTORIA ELYMI Ell. and Ev. occurs in fuscous lesions on *Agropyron subsecundum* at the end of the road to the Snow Bowl out of Flagstaff, Arizona. The host, which was growing in woods, resembled *Elymus glaucus* Buckl. except for the definite *Agropyron* arrangement of the spikelets. The fungus is new on this host from the Southwest. The spores are whiplike, curved, pointed sharply at the apex and enlarged slightly near the base, 32–57 × 1.2–1.6 μ, 3-septate, clear hyaline, borne in pycnidia up to 200 μ diameter (WSP 46012). This material is similar to *S. elymi* reported on *A. trachycaulum* from the same general region (**17**) although the spores are narrower on *A. subsecundum*. *Septoria elymi* also occurs on leaves of *Elymus canadensis* L. in Pump

House Wash in the Oak Creek Canyon area, Arizona (WSP 46116). The spores are small, 20–37 × 1.0–1.5 μ, mostly nonseptate or 1-septate. Associated with them are some small pycnidia (50–60 μ) containing masses of arcuate, nonseptate microspores, 6–10 × 0.6–1.0 μ. These appear to be obvious microspores and are not *Selenophoma*. They appear to be the same as *Septoria gracilis* Pass. or possibly *S. cristata* Hollós (**6**). I suspect that these are microspores of *S. elymi* produced under summer conditions of erratic rainfall when most microspores of *Septoria* spp. are formed. Their small size and great numbers are typical of microspores. The crescent shape is unusual but is similar to the microspore stage of *S. tritici* except that the latter lacks the sharply pointed crescent ends seen in the Pump House Wash material. It should be added that the small pycnidia of the microspores are intimately associated in well defined lesions with the macrospore-containing pycnidia.

In addition to the microspores and macrospores of *S. elymi* a few larger spores, 25–40 × 2.5–2.8 μ with three septations were seen, apparently from scattered pycnidia of SEPTORIA AGROPYRINA Lobik (also in WSP 46116) found in older leaf tissue invaded by secondary fungi.

SEPTORIA AVENAE Frank which was almost omnipresent on *Glyceria* spp. in the West in 1957 was new to Utah on *G. grandis* S. Wats. along a creek in the Wasatch National Forest, 13 miles east of Kamas (WSP 46118). It was also found on *G. striata* (Lam.) Hitchc. along a creek 18 miles east of Huntsville, Utah (WSP 46071). Recently I reported material on *Bromus* spp. which was close to, if not identical with, *S. avenae* (**17**). I have another fragment, this time on *B. carinatus* (Hook.) Arn. from Salmon Arm, B. C., which seems to be *S. avenae* (WSP 46237). The narrowly cylindrical spores are sometimes curved, blunt at each end, 45–60 × 3.5 μ, nonseptate or faintly triseptate. The fungus is virtually saprophytic, thriving on nearly dead grass in an orchard following prolonged rains. It was associated with leaf rust, *Ovularia, Selenophoma bromigena* and various saprophytes. Some material collected by A. D. E. Elmer in the Olympic Mountains in August, 1900 on *Bromus ciliatus* L. is, however, referred to STAGONOSPORA BROMI Sm. and Ramsb. The old material of Elmer shows chocolate brown lesions and small spores 14–21 × 2.6–3.0 μ, triseptate, hyaline and fusiform-cylindric. Some chocolate spots with buff centers occur on *B. laevipes* Shear found along the shore of Clear Lake, California (WSP 42264). The spores were immature, mostly 1-septate and up to 20 μ long. The pycnidia were globose, ostiolate, golden brown, about 160 μ diameter. We have assigned this to *St. bromi* also. Associated in this phanerogamic specimen were separate leaves with many promi-

nent pycnidia typical of SEPTORIA JACULELLA Sprague (cf **11**). This was filed separately as WSP 42263. There is also a fragment on *Bromus marginatus* Nees collected by Frank W. Gould in Cochise County, Arizona, August 27, 1944 (WSP 42266) which has large pale pycnidia and 1–3-septate spores. The material was badly damaged by heat so that details of the spores were lacking. A few that were separable from the collapsed spore mass were recognizable as *St. bromi*. We have a fragment on *B. sitchensis* from Friday Harbor, Washington (WSP 42283) which consists of one buff spot with a broad fuscous border containing 2 large pycnidia. The spores are fusoid-pointed at the apex, blunt at the base, 3-septate, 25–35 × 3.0–3.5 μ. This we call *Septoria avenae*. *St. bromi* was also noted on a phanerogamic herbarium sheet of *B. purgans* L. from Tate City, Georgia, collected by A. Cronquist (WSP 42270).

PHYLLOSTICTA sp. with tiny bacillar to comma-shaped spores (4.6 × 0.5–0.8 μ) in scattered pycnidia was found sparingly on necrotic leaves of *Bromus ciliatus* at Perth Springs, Nevada. The spores are probably microspores of a large-spored fungus and are not described as new. They are obviously distinct from those of *P. bromivora* Sprague (cf **14**, p. 551–552 and fig. 1, C). This material was taken from a phanerogamic specimen collected by E. T. and S. H. Harper, August 15, 1896 (WSP 42259). The same kind of microspores was found on *B. sitchensis* at Barkley Sound, B. C. (WSP 42278) collected by J. R. Anderson (886) July, 1918.

In a recent article I mentioned summer material of *Septoria tenella* Cke. and Ellis in woods on *Festuca subulata* Trin. in Okanogan County, Washington (**16**). I have similar material from a comparable habitat in the same latitude in Bonner County, Idaho, above Goose Creek (WSP 46085). The 0-septate spores are 13–20 × 1.0–1.2 μ, straight, cylindrical, or slightly larger at one end. A few spores showed faint indications of a central septum.

SEPTORIA SPARTINAE (Trel.) Sprague occurred on *Spartina gracilis* Trin. at Knutsford, B. C. (WSP 46227).

SEPTORIA INFUSCANS (Ell. & Ev.) Sprague was common in the Okanagan Valley, B. C. on *Elymus cinereus* (cf WSP 46231, Knutsford and WSP 46245, Penticton).

SEPTORIA JACULELLA Sprague occurs on *Bromus pacificus* Shear at Tillamook Point, Oregon. It was collected by W. J. Spillman August 10, 1899 (WSP 42271). Although this was collected over 60 years ago, the fungus is unreported on this species of *Bromus*. The compact

black pycnidia contain only a few spores. The pycnidia are the thick-walled kind so often seen in seacoast collections of the Sphaeropsidales.

ASCOCHYTA SORGHI Sacc. occurs on dried leaves of *Bromus mollis* L. collected at Pullman, Washington, June 21, 1901 by C. V. Piper (WSP 42276). The material is badly plasmolyzed but all spores that were seen were 1-septate. No report of *Ascochyta* on *B. mollis* is available from the western area. A phanerogamic herbarium specimen of *A. sorghi* on *B. commutatus* Schrad. was made by H. A. Wahl in Huntingdon County, Pennsylvania (WSP 42255).

SEPTORIA OUDEMANSII Sacc. caused an eyespot on leaves of *Poa longiligula* Scribn. and Will. in dry woods in Oak Creek Canyon, Arizona, September 1, 1957 (WSP 46293). In my recent paper in this series I reported *S. oudemansii* on *P. pratensis* from the same place but the material on *P. longiligula* was not determined at that time. While the collection on *P. pratensis* had the typical spores of *S. oudemansii*, the one I have from *P. longiligula* has slightly different spores. They measure 16–19 × 2.7–3.0 μ and are somewhat less symmetrical than typical of this species. One end is frequently less tapering than the other, more like young spores of *S. nodorum* Berk. Material from this area should be studied in pure culture. Until this can be done the specimen is assignable to *S. oudemansii*.

J. C. Lindquist, Institute Spegazzini, La Plata, Argentina, has very kindly sent several specimens of *Septoria* to me for examination. One is the TYPE of SEPTORIA TRISETI Speg. and also of SPHAERELLA TRISETI Speg. The *Sphaerella* is a species of *Mycosphaerella*. The host is now known as *Koeleria phleoides* (Vill.) Pers. I was not able to find the *Septoria* in the mouldy leaf fragment seen. There is an old sketch on the inner packet showing a habit sketch of pycnidia and a drawing of a needlelike spore about 25 × 1 μ. In our earlier emendation of *S. triseti* we restricted the emendation to *Agrostis* (**11**). The fungus differs appreciably from *S. calamagrostis* in its much smaller spores and pycnidia. Lindquist also sent a recently collected specimen on *K. phleoides* which he, in my opinion, correctly determined as *Septoria calamagrostidis f. koeleriae* (Cocc. and Mor.) Sprague (Inst. Speg. 21835). It has large, light brown pycnidia 90–120 × 140–200 μ in prominent white spots with buff borders (FIG. 1, A). The spores are like those in western North America, needlelike or stiffly curved, 35–60 × 1–1.3 μ. This would tend to verify our assumption that the *Septoria* on *Koeleria* from Argentina described by Spegazzini on *Koeleria* was *S. calamagrostidis f. koeleriae*. Therefore *S. triseti*, in spite of its name, is recognized only

on *Agrostis*. In my opinion the habit sketch, apparently Spegazzini, strongly suggests stunted material of *S. calamagrostidis f. koeleriae* indicating that the type of *S. triseti* belongs under the fungus Cocconi and Morini described earlier (cf **3**, **10**). In the continued absence of the material on *Agrostis magellanica* Lam. and on the basis of the above observations *S. triseti* Speg. em. Sprague is a *nomen dubium* or at most *nomen confusum*. It now seems desirable to recognize the material on *Agrostis* from the western United States with small spores as an undescribed species distinct from *S. calamagrostidis*. I therefore propose:

Septoria agrosticola sp. nov.

Maculis indeterminatis, sublinearibus v. irregularibus, cinereis; pycnidiis obscuribus, nigro-brunneis, ostiolatis, substomatalibus, globosis v. subglobosis, 40–100 (–120) μ diam., pycnophoris 2.5–3.5 μ; pycnosporulis filiformibus, v. subbacillaribus, rectis, flexis, v. curvulis, 0–1-septatis, 16–43(–18–35) × 0.8–2.1(–1.3–1.7) μ.

Lesions on leaves indeterminate, sublinear to irregular, or on leaf tips, cinereous; pycnidia not prominent, black-brown, ostiolate, substomatal, globose to flattened at the ostiolar end where it is appressed against the leaf epidermis, 40–100(–120) μ (mostly 40–80 μ) in diameter, formed of irregular, oblong-polyhedral, parenchymatous cells, in strands, which converge in radii towards the ostiole, brown outer cells 2 layers thick (3 μ) with 2 or 3 inner hyaline layers (3–5 μ thick) giving rise to narrowly bulbous-cylindrical pycnophores, 2.5–3.5 μ; pycnospores filiform to subbacillar or sometimes narrowly fusiform, straight, bent or less often moderately curved, 0–1-septate, 16–43(–18–35) × 0.8–2.1 (–1.3–1.7) μ.

HOLOTYPE: On *Agrostis tenuis* Sibth., Alsea Valley, Benton County, Oregon, November 6, 1938, leg. *W. B. Cooke*, OSC 274.

PARATYPES: On *Agrostis alba* L.: Springdale, Oregon, March 13, 1939, *R. Sprague*, OSC 432; Fort Steilacoom, Washington, April 11, 1939, *Sprague*, OSC 583; Waldport, Oregon, September 24, 1939, *Sprague* OSC 731; near Corvallis, Oregon, March 6, 1940, *Sprague*, OSC 798; Corvallis, Oregon from artificial inoculation of culture 113 (Shedd, Oregon), December–February 1939–1940, *Sprague* OSC 878. On *A. exarata* Trin. var. *ampla* (Hitchc.) Hitchc.: Lewisburg, Oregon, June 13, 1939, *Sprague*, OSC 647. On *A. tenuis* Sibth.: Corvallis, Oregon, February 8, 1938, *Sprague*, OSC 43; Corvallis, Oregon, February 22, 1938, *Sprague*, OSC 80; near Barview, Oregon, March 8, 1938, *Sprague*, OSC 151; 4½ miles east of Hebo, Oregon, March 7, 1938, *Sprague*, OSC 155; near Young's Bay, Oregon, November 17, 1938, *Sprague*, OSC 365; Corvallis, Oregon, February 2, 1939, *Sprague*,

OSC 368; 7 miles west of St. Helens, Oregon, March 15, 1939, *Sprague*, OSC 430; Astoria Airport, Clatsop County, Oregon, March 16, 1939, *Sprague*, OSC 431; Young's River, Clatsop County, Oregon, March 16, 1939, *Sprague*, OSC 433; Comstock, Oregon, March 7, 1939, *Sprague* OSC 434; 3 miles south of Chehalis, Lewis County, Washington, April 12, 1939, *Sprague*, OSC 576; 3 miles south of Kelso, Cowlitz County, Washington, April 12, 1939, *Sprague*, OSC 578; Mary's Corner near old courthouse, Lewis County, Washington, April 12, 1939, *Sprague*, OSC 579; 3 miles south of Tenino, Washington, on prairie along U. S. Highway 99, April 12, 1939, *Sprague*, OSC 582; Tenino, Washington, April 12, 1939, *Sprague*, OSC 587; Barview, Tillamook County, Oregon, May 12, 1939, *Sprague*, OSC 8380-A; 2½ miles west of Nashville, Lincoln County, Oregon, February 21, 1938, *Sprague*, OSC 8481; Corvallis, Oregon, January, 1935, *Sprague*, OSC 10417; Corvallis, Oregon, February 2, 1936, *Sprague*, OSC 10483; Corvallis, Oregon, January 29, 1936, *Sprague*, OSC 10484. On *A. "tenuis* × *A. palustris"* (field hybrid): 6 miles south of Newport, Oregon, December 19, 1937, *W. B. Cooke*, OSC 16. This is probably *A. stolonifera* L. The holotype and the numerous paratypes are filed in the Department of Botany, Herbarium, Oregon State College, Corvallis (OSC), Dr. Albert N. Steward,[5] curator. *S. agrosticola* was once found on *A. castellana* Boiss. and Reut. an exotic growing in the grass nursery near (Granger) Corvallis, Oregon. *S. agrosticola* is confined to the humid coastal portions of Oregon and Washington, west of the Cascade Mountains.

Lindquist also sent material of SEPTORIA BROMI Sacc. on *Bromus mollis* from Quilmes, Parque Pereyra Iroala (Inst. Speg. 26667). The spores had the typical whiplike shape that I find in this species in western North America (11). I also have *S. bromi* on herbarium fragments as follows: on *B. purgans*, State College, Pa. (WSP 42269); *B. secalinus* L., Cannon Falls, Minn. (WSP 42279); *B. ciliatus* L., Itasca Park, Minn. (WSP 42258).

Some years ago I discussed SEPTORIA NODORUM Berk. on woodland grass, *Festuca subulata* from Okanogan County, Washington (11, p. 245). It had spores 21–30 × 3.1–4.8 μ. In 1957 I found similar material in Pend Oreille County, Washington with 1-septate, narrowly cylindrical spores 12–19 × 2.5–3.2 μ. These could be called *Ascochyta sorghi* Sacc. but the brown blight symptoms, the shape of the spores, more pointed at one end than the other and their outline indicates that this is summer material of *S. nodorum* (WSP 46108). Somewhat

[5] Deceased.

similar material was seen causing a severe blotch over most of the culms, heads and leaves of *Agropyron spicatum* (WSP 46240), and *A. inerme* (Scribn. and Sm.) Rydb. (WSP 46241) in Tumwater Canyon along the Wenatchee River, Chelan County, Washington, September 30, 1958. The spores were like *A. sorghi* Sacc., mostly 14–24 × 2.5–3.0 μ but some were 2–3-septate. The symptoms were typical of *S. nodorum*. The fungus was severe enough to be a limiting factor in the development of the *Agropyron* grasses growing at the base of steep slopes in this wooded narrow river valley. *A. repens* showed only traces of the fungus in the vicinity.

STAGONOSPORA SIMPLICIOR Sacc. and Berl. occurs on *Stipa comata* Trin. and Rupr. in Lees Canyon, Nevada (WSP 46076). Associated on the same host is *Scolecotrichum graminis* Fckl. (WSP 46077). *St. simplicior* caused a few prominent lesions on *Stipa columbiana* Macoun in Lees Canyon (WSP 46124).

DARLUCA FILUM (Biv.) Cast. occurs as a parasite on *Puccinia sessilis* Schm. on *Phalaris arundinacea* L. at Huntsville, Utah. Some associated phragmospores appear to be young stages of *Hendersonia culmicola* Sacc.

A small amount of COLLETOTRICHUM GRAMINICOLA (Ces.) Wils. was found on old leaves of *Glyceria pauciflora* Presl in a meadow along Goose Creek, Bonner County, Idaho (WSP 46084). *C. graminicola* occurs on *Bromus kalmii* A. Gray collected by J. B. Moyle at Itasca Park, Minnesota (WSP 42265). This is new, at least to the western United States. The fungus was found by C. G. Shaw on *B. ciliatus* at Mendota, Wisconsin (WSP 42256), and on *B. purgans* at Waco, Texas, by V. L. Cory (WSP 42231).

RHYNCHOSPORIUM SECALIS (Oud.) J. J. Davis occurs on sheaths and leaf bases of *Hordeum brachyantherum* Nevski along a mountain meadow stream near Aspen Mirror Lake, Highway 14, Utah (WSP 46095). The fungus is uncommon on this host. *R. secalis* was abundant on *Bromus ciliatus* growing under aspens along Snow Bowl Road out of Flagstaff, Arizona (WSP 46109) and a fragment of it was found on the same host near Buffalo, Wyoming (WSP 42257).

OVULARIA PUSILLA (Ung.) Sacc. and D. Sacc. causes a yellowish to fawn colored subcircular spot on *Muhlenbergia filiformis* (Thurb.) Rydb. growing in brush on Tioga Pass, California (WSP 46037). Only a few young spores were found on the leaves. There is no report of *Ovularia* on *Muhlenbergia*. The same fungus causes buff eyespots on *Trisetum spicatum* (L.) Richt. at Mirror Lake, 35 miles east of Kamas, Utah (WSP 46066). *O. pusilla* occurs on *Bromus carinatus* at Salmon

Arm, B. C. (WSP 46236). *O. pusilla* caused a buff leafspot on *B. orcuttianus* Vasey. It was collected by William Cusick at 6000 ft elev., above the Imnaha River in Wallowa Mts., Oregon (WSP 42277) August 10, 1909. This is an unreported host for the organism. An illustration of *O. pusilla* on *Agrostis alba* from Blickenderfer Creek, Washington (WSP 46067) is shown in FIG. 1, C.

HENDERSONIA (Wojnowicia) GRAMINIS McAlp. produces prominent pycnidia on basal sheaths of *Muhlenbergia filiculmis* Vasey near Navajo Lake on State Highway 14, Utah (WSP 46054). There is no report of a fungus on this grass.

SEPTOGLOEUM OXYSPORUM Sacc., Bomm. and Rouss. was prevalent on *Agrostis exarata* in brush along the West Branch of Priest River, Pend Oreille County, Washington (WSP 46055). Char spot has been reported only from Wyoming on this host.

SCOLECOTRICHUM GRAMINIS Fckl. caused heavy damage on leaves of *Hordeum brachyantherum* along a creek at Aspen Mirror Lake, Utah (WSP 46049) and on the same host in Midway Valley (WSP 46052) nearby. It produced a very prominent brown streak on leaves of *Glyceria grandis* at Huntsville, Utah (WSP 46051). It occurred with immature perithecia on *Poa canbyi* (Scribn.) Piper at base of Bald Mountain, Utah (WSP 46065). The fungus was present in trace amounts on living leaves of *Melica subulata* (Griseb.) Scribn. in Pend Oreille County, Washington (WSP 46107). It was found on *Poa gracillima* in the Blewett Pass area, Chelan County, Washington associated with *Puccinia poae-sudeticae* (Westd.) Jørstad. (WSP 46210). *S. graminis* occurs on a phanerogamic herbarium specimen of *Bromus sitchensis* collected by J. K. Henry at Crescent, B. C. (WSP 42282) and on the same host at Barkley Sound, B. C. by J. R. Anderson (WSP 42284). It was developing the ascigerous stage on leaves of *Poa secunda* Presl at Mirror Lake Forest Camp east of Kamas, Utah (WSP 46290).

HETEROSPORIUM PHLEI Gregory produced abundant spots on *Phleum alpinum* L. in the Plant Introduction Station Nursery, Pullman, Washington (WSP 46135). The material was collected by Sherl M. Dietz. As is the usual case, spores were scarce.

FUSARIUM AVENACEUM (Fr.) Sacc. and other *Fusarium* spp. occur on blighted heads of *Hesperochloa kingii* (S. Wats.) Rydb. in Deer Valley, an area near State Highway 14 below Cedar City, Utah. The head injury, however, seems to be mostly due to a bacterial slime disease, possibly *Corynebacterium*. Masses of bacteria occur in the glumes and spikelet parts (WSP 46043). I have not seen a comparable condition on this host previously.

FUSARIUM POAE (Pk.) Wr. caused a buff leaf rot on *Sitanion hystrix* in Pump House Wash in Oak Creek Canyon, Arizona (WSP 46074). Both pip-shaped microspores and 1-septate falcate spores were present in clusters which protruded in seried rows from the stomata. The fungus is unreported on *Sitanion*.

FUSARIUM GRAMINEARUM Schwabe occurs on *Bromus purgans* at Forest Mills, Minnesota, on WS 2699 in the phanerogamic collections at Washington State University. BIPOLARIS (Helminthosporium) SOROKINIANUM (Sacc. in Sorok.) Shoemaker occurs with it.

A mold on dried plants of *Agropyron riparium* Scribn. and Sm. at Pilot Butte, Oregon, proved to be mostly moldy last year's pustules of *Puccinia recondita* Roberge ex Desm. (*P. rubigo-vera*) unreported from Oregon on this dry-land grass (WSP 46003). The spores were extremely long and slender.

PUCCINIA POAE-SUDETICAE (Westd.) Jørstad caused a red rust spotting on leaves of *Trisetum spicatum* along the rim of Crater Lake, Oregon (WSP 46125). The comma-shaped paraphyses were abundant.

There appears to be no report of *Erysiphe graminis* DC. ex Merát on *Agropyron trachycaulum* from Arizona. It was common on the leaves and heads of this grass toward the upper end of the Snow Bowl out of Flagstaff, Arizona (WSP 46093).

Some head blight material of EPICHLOE TYPHINA (Fr.) Tul. on *A. trachycaulum* was given to me by Sherl M. Dietz from the Plant Introduction Station Nursery, Pullman, Washington (WSP 46311). An illustration of this material is shown (FIG. 2, A, B). The nursery-row material (232-160, Utah) had been infected in 1957 and again in 1958. This systemic fungus is seed-borne (cf **13**) and probably the fungus originated from Utah, an unreported area.

PIRICULARIA GRISEA (Cke.) Sacc. was found on *Leersia oryzoides* (L.) Swartz (WSP 46171) at Bloomington, Indiana, August 26, 1958. Apparently unreported from Indiana on this grass the specimen is of interest because it brings our attention to *Dactylaria graminum* (Schw.) Sacc. (**7**, *4:* 195; *13:* 330). The type of *Dactylium graminum* Schw. is not yet available. I suspect that it is immature material of the fungus generally known as *P. grisea*. Schweinitz described *Dactylium graminum* a number of years before Cooke described *Trichothecium griseum* (*P. grisea*) (**4**, **7**).

Associated with the last-mentioned material was an abundance of BIPOLARIS LEERSII (Atk.) Shoemaker on *Leersia virginica* Willd. (WSP 46173), apparently unreported from the region. In the same marshy area along Jordan Creek on the Campus of the University of Indiana

I collected some material of *Glyceria striata* which showed buff elliptical lesions comparable to those caused by *Septoria avenae* Frank. The only fungus present consisted of tiny pycnidia 30–40 μ diameter containing bacillar microspores 6.8–7.6 × 0.8 μ (WSP 46174). These are probably microspores of *S. avenae*.

MYCOSPHAERELLA MAYDIS (Pass.) Lindau was collected by Juan B. Ferrer on *Digitaria decumbens* Stent. in Panama. He sent one heavily infested leaf by airmail April 24, 1958. As shown by the illustration (FIG. 1, B), the somewhat gregarious perithecia are numerous. They are black, depressed-globose, 40–100 μ diameter with a prominent ostiole, 14–21 μ diameter. The material is badly plasmolyzed and determination of the contents of the fruiting body was very difficult. The asci appear to be about 27–30 × 9–10 μ and are without evidence of any paraphyses. The spores are yellowish, 14–18 × 3–4 μ with the top cell larger than the lower one. Weiss listed *M. maydis* as the cause of a leafspot in Puerto Rico (**20**). The original description (cf **7**, *1*: 525) does not indicate that *M. maydis* was associated with any definite leafspot. For the present, at least, this collection can be assigned to *M. maydis*.

DOTHIDELLA ARISTIDAE (Schw.) Ell. and Ev. is widespread on *Distichlis stricta* (Torr.) A. Gray in eastern British Columbia (cf WSP 46234, 9 miles east of Kamloops).

A number of grass collections from Dade County, Florida, was made by Shirl Graham in July, 1956 and given to me for study of associated fungi. A few of these are of interest: PHYLLACHORA ANDROPOGONI Ell. and Ev. occurred on *Andropogon elliottii* Chapm. near Perrine, Florida (WSP 46258). P. GUIANENSIS Stev. caused a circular to subcircular tar spot on *Paspalum longepedunculatum* Le Conte at Palm Springs, Florida (WSP 46263). A trace of PHYLLOSTICTA SORGHINA Sacc. was found on *Rhynchelytrum roseum* (Nees) Stapf and Hubb. from South Miami. MELIOLA (?) PANICI Earle was abundant on dead leaves of *Chloris glauca* (Chapm.) Wood at Perrine (WSP 46259). A rust on leaves of *Andropogon hirtiflorus* (Nees) Kunth at South Miami was assigned to *Puccinia andropogonis* Schw. but clavate paraphyses were common at the margins of the obscure pustules (WSP 46261). *Colletotrichum falcatum* Went was, of course, common on a number of hosts including *Sorghum sudanense* (Piper) Stapf which I do not have listed. It is undoubtedly omnipresent on this host. Graham collected it at the Subtropical Experiment Station, Homestead (WSP 46260).

One of the molds on dead leaves of *Rhynchelytrum roseum* was a pycnidial form exuding long brown tendrils in water mounts. The small guttulate, cylindrical spores do not fit *Sphaeropsis*. I compared this

fungus with *Microsphaeropsis* v. Höhnel also. The description of *M. bakeri* Syd. (7, *25: 260*) indicates that our fungus is similar to *M. bakeri*. However, v. Höhnel based his genus on *Coniothyrium olivaceum* Bon. apud Fuckel which has ellipsoidal spores. Clements and Shear place *Microsphaeropsis* under *Coniothyrium* (2). Therefore the information available indicates that our fungus does not belong to the dubious genus *Microsphaeropsis*. In using Bender's key to the Sphaeropsidales I am unable to classify this fungus. It keys to *Sclerosphaeropsis* Bubak (1) which is another genus considered synonymous with *Coniothyrium* by Clements and Shear (2). There does not seem to be any genus with brown *Phyllosticta*-like spores. Since the spores have the wrong outline for either *Sphaeropsis* or *Coniothyrium*, it must be considered either as a member of an undescribed genus of the Amerosporae-Phaeosporae or an immature member of the brown spored Phragmosporae, such as *Hendersonia*. Because of the small quantity of material available for study, I prefer to place it in *Hendersonia* sp. A brief description is given, however, as follows: in dry foliage, pycnidia brown, ellipsoid, erumpent, ostiolate, 190–250 × 115–145 μ; pycnidiospores cylindrical to fusiform-cylindrical, brown, copious, multiguttulate (pseudoseptate) 10–14 × 1.9–2.4 μ. South Miami, Florida, July 3, 1956 (WSP 46262).

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THE OCCURRENCE OF AMINO ACIDS IN FOUR SPECIES OF PYTHIUM

RICHARD B. HINE[1]

The use of chemical techniques as an aid in taxonomy has received considerable attention in the last few years. Paper chromatography has been used by many workers to determine quantitative or qualitative differences, or both, in fungi (**1**, **2**, **3**, **4**).

The object of this work was to determine if differences existed in the free amino acid content between isolates of *Pythium aphanidermatum* (Edson) Fitzpatrick, *Pythium arrhenomanes* Drechsler, *Pythium debaryanum* Hesse, and *Pythium ultimum* Trow.

MATERIALS AND METHODS

One hundred ml of the following medium was dispensed into 250 ml Erlenmeyer flasks and sterilized by filtration:

$NaNO_3$	2.0 g
$K_2HPO_4 \cdot 3H_2O$	1.0 g
KCl	0.50 g
$MgSO_4 \cdot 7H_2O$	0.50 g
0.5% $Fe_2(SO_4)_3$	0.4 ml
glucose	10 g
distilled water	to 1 liter

The resultant pH was 6.3.

A hyphal tip of actively growing mycelium from water agar cultures was placed in each flask. Ten flasks were inoculated with one isolate of each fungus. The flasks were capped with filter paper rather than cotton plugs to prevent the introduction of cotton fibers. The stationary cultures were incubated for 10 days at 25° C. Mycelium from the replicate cultures of each fungus was pooled, filtered, and washed with 100 ml of distilled water to remove traces of the medium. The mycelium was immersed in hot 95% ethanol for 10 minutes. The supernatant was decanted and saved. The mycelium was homogenized and extracted with 50% ethanol until all uncombined amino acids were

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removed. The ethanol extracts were then desalted with cation exchange resin IR-120. The water washings and the NH_4OH eluates of the resin were combined and evaporated to dryness on a steam bath. The amino acid residue was taken up in 5 ml of 20% ethanol and samples stored at 4° C.

Two dimensional chromatograms spotted with 150 μl of the extract were made on sheets of Whatman #1 filter paper (15 × 18 in.). Chromatograms of a known mixture of amino acids were used as standards. The papers were developed simultaneously with N-butyl alcohol, acetic acid, and water in the ratio of 6.3:1:2.7 respectively for 24 hours and dried in a steam heated oven for 1 hour. The papers were then developed for 24 hours in a second direction using 80% redistilled phenol in an ammonia atmosphere. After drying the chromatograms in a steam heated oven for 1 hour, the papers were sprayed with 0.3% ninhydrin solution. Density of the spots was determined by use of a photovolt densitometer. Readings were made on the papers after they had been in a dark cabinet for 24 hours.

RESULTS

P. ultimum, *P. debaryanum*, *P. aphanidermatum*, and *P. arrhenomanes* grew rapidly in the basic medium.

TABLE I

OCCURRENCE AND RELATIVE CONCENTRATIONS OF AMINO ACIDS IN FOUR SPECIES OF PYTHIUM

Amino acid	*Pythium ultimum*	*Pythium arrhenomanes*	*Pythium debaryanum*	*Pythium aphanidermatum*
Aspartic acid	0.12*	0.05	0.19	0.03
Glutamic acid	0.60	0.85	1.12	0.52
Serine	0.48	0.32	0.50	0.33
Glycine	0.38	0.19	0.30	0.12
Alpha-alanine	1.72	1.00	1.45	1.48
Asparagine	0.10	—	0.15	—
Glutamine	0.15	—	0.12	—
Lysine	1.70	1.35	1.36	1.17
Arginine	0.89	0.50	0.85	0.52
Tyrosine	0.10	—	0.15	0.13
Gamma-amino butyric acid	0.32	0.58	0.55	0.16
Valine	0.90	0.17	0.60	0.24
Leucine	0.15	0.14	0.19	0.12
Cysteine	0.07	0.03	0.07	0.01
Proline	—	0.30	—	0.18
Unknown 1			0.14	
Unknown 2		0.10		

* Optical density readings determined on a photovolt densitometer.

The following amino acids were produced by all 4 species of *Pythium:* aspartic acid, glutamic acid, serine, glycine, alpha-alanine, lysine, arginine, gamma-amino butyric acid, valine, leucine and cysteine.

P. arrhenomenes and *P. aphanidermatum* did not produce detectable asparagine or glutamic acid but were the only species for which proline was detected. These species differed from each other in that tyrosine was not detected in mycelium of *P. arrhenomanes.*

P. ultimum and *P. debaryanum* did not produce detectable proline and differed from each other, besides certain quantitative differences, in that an unknown spot was produced by *P. debaryanum.* Quantitative differences of the amino acids did not seem to be very important for the 4 species except in gamma-amino butyric acid, valine, and glutamic acid. These data are summarized in TABLE I.

CONCLUSIONS

Fifteen amino acids have been shown to be synthesized by species of the genus *Pythium* when grown on a medium with only glucose as a carbon source and NO_3-N as a nitrogen source. There are qualitative and quantitative differences between the 4 species studied. Further studies need to be made to determine if there is as much variation between isolates of the same species as between different species in the genus before the practicability of using these differences as a taxonomic tool could be ascertained.

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NEW RECORDS OF FOREST FUNGI IN THE SOUTHWEST

WALLACE E. ESLYN [1]

A number of forest fungi have been collected since September 1957 that either have not been reported previously in Arizona or New Mexico or have not been recorded in these States on their present hosts. Although two of the latter, i.e., yucca and cholla, included in this report are not considered forest species, they are at times associated with pinyon and juniper of the woodland forest.

Favorable moisture conditions during 1957, following a long period of drought in the Southwest, were probably responsible for the appearance of some of these fungi. Weather records for New Mexico indicate that 1957 was the wettest year since 1941 (**4**), while during the same period most of Arizona received above-average precipitation (**4**).

The fungi reported here have been deposited in the herbarium of the Forest Disease Laboratory at Albuquerque, New Mexico. They are arranged by hosts and described as follows:

ON POPULUS TREMULOIDES MICHX.

1. *Daldinia occidentale* Child

Stromata hemispherical, carbonaceous, sessile, 2.8–7.3 × 2.3–6.3 × 0.8–3.7 cm; perithecia 900–1275 × 375–540 μ, monostichous; spores 11.3–14.8 × 7.1–8.1 (–9.9) μ, brown, ellipsoid to inequilaterally ellipsoid.

On lower trunk of living aspen, Munds Park Road, Coconino National Forest, Ariz., June 26, 1958, *S. R. Andrews, J. S. Lieberman, and W. E. Eslyn.*

Child (**1**) records this species as being found on dead *P. tremuloides* in Colo. Not previously recorded on this host in Ariz.

2. *Valsa translucens* De Not.

Stromata about 1–1.2 mm wide, locules approximately 194–318 μ wide × 175 μ high; asci 35–53 × 8.8–14.0 μ; spores 7.1–11.3 × 2.1–2.8 μ.

[1] Plant Pathologist, Rocky Mountain Forest and Range Experiment Station, Forest Service, U. S. Department of Agriculture. General headquarters at Colorado State University in Fort Collins, Colorado. Research reported here was completed at the Station's Forest Disease Laboratory at Albuquerque, New Mexico.

On bark of declining aspen, Beartrap Canyon, Cibola National Forest, N. Mex., Nov. 9, 1958, *W. E. Eslyn.* Apparently the first report of this fungus on aspen in N. Mex.

3. *Cytospora translucens* Sacc.

The imperfect stage of *Valsa translucens.* Spores slightly larger than described, 4.5–6.4 × 1.0–1.8 μ; conidiophores 10.6 × 1.4 μ. Found both solitary and sharing the same stroma as the *Valsa* stage.

Collected on same tree as above.

ON POPULUS ANGUSTIFOLIA JAMES

1. *Teichospora oblongispora* Ell. & Ev.

Perithecia erumpent, black, collabent, papillate, 300–420 × 225–300 μ; asci clavate-cylindrical, 60–95 × 15.2–21.2 μ, paraphysate, 8-spored; spores brown, muriform, 5–7 (generally 5) septate, indented at the septa; with one or more cells divided by longitudinal septa, 21.2–31.8 × 9.9–10.9 μ.

On dead twigs of declining mountain cottonwood, Bishop Lodge Road, near Tesuque, N. Mex., March 18, 1959, *W. E. Eslyn.*

Previously reported on dead twigs of this host from Fort Collins, Colo. Apparently not previously recorded from N. Mex.

2. *Teichospora populi* (Earle) Sacc. & D. Sacc.

Perithecia erumpent, black, subglobose, 255–450 × 180–390 μ, ostiole papilliform to simple and flattened; asci substipitate, 76–95 × 11.2–14.1 μ, increasing in length in distilled water to 176 μ; spores brown, 3-septate (occasionally 4 or more), with 2 or more longitudinal septa.

On decorticated branch of mountain cottonwood, Bishop Lodge Road, near Tesuque, N. Mex., March 18, 1959, *W. E. Eslyn.*

Previously reported on decorticated twigs of this host at Durango, Colo. Apparently not reported before in N. Mex.

3. *Eutypella radula* (Pers. ex Fr.) Ell. & Ev.

Perithecia 525–705 × 375–600 μ, subglobose, ostioles sulcate, erumpent, densely scattered within an extensive whitish stroma; producing a mottled pattern upon the interior surface of the bark and upon the wood surface underlying the stroma; asci long-stalked, 63–116 × 10.6 μ including stalk; paraphyses abundant, densely guttulate, filiform, about

176–240 μ long; spores allantoid, light brown, 8.1–14.1(–14.8) × 2.4–3.2 μ.

Cf. Ell. & Ev. Fungi Columbiana 2025. The sporidial length has been given as 10–12 μ for European specimens; however, in the exsiccati examined the spores were 7.4–14.1 × 2.2–3.2 μ.

On bark of small branch of declining mountain cottonwood, Bishop Lodge Road, near Tesuque, N. Mex., March 18, 1959, *W. E. Eslyn.* Apparently a new host record for this fungus.

4. *Cytospora ambiens* Sacc.

Loculi several or merged into one irregular chamber, stroma up to 2 mm wide; conidiophores branched, whorled at the base, 14.1–17.7 μ long; spores hyaline, allantoid, 3.9–6.4(–7.1) × 1.0 μ, oozing out as a small whitish mass when wet.

On bark of large dead branches of declining mountain cottonwood, Bishop Lodge Road, near Santa Fe, N. Mex., Aug. 27, 1958, *J. S. Lieberman and W. E. Eslyn.*

Apparently a new host record for this fungus.

A *Valsa* was noted on other branches, which externally fitted the description of *V. ambiens;* however, no asci or spores were found to verify this determination.

ON ACER NEGUNDO L.

1. *Phyllactinia guttata* Lév.

Cleistothecia 216–245 μ diam, appendages 191–290 μ long; asci 72–83 × 32–40 μ; spores 2 per ascus, 31–36 × 21–25 μ.

On lower side of leaves of wilting boxelders, near Sandia Ranger Station, Manzano Mts., Cibola National Forest, N. Mex., Sept. 23, 1957, *S. R. Andrews and W. E. Eslyn.*

Apparently not previously reported on this host in N. Mex.

2. *Tubercularia vulgaris* Tode ex Fr.

Spores 6.5–8.3(–10.8) × 2.5–3.6 μ.

On bark of boxelder, near Sandia Ranger Station, N. Mex., Sept. 23, 1957, *S. R. Andrews and W. E. Eslyn.*

Previously reported by Fairman (2) on *Salix* sp. from N. Mex.; however, not found prior to this on boxelder in N. Mex.

ON DUFF

1. *Leucopaxillus amarus* (Alb. & Schw.) Kuehner f. *bicolor* (Murrill) Singer & A. H. Smith

Spores hyaline, containing usually one large vacuole, amyloid, 4.7–7.2 × 4.7 μ; basidia 5.4–7.2 μ in width.

This specimen was identified by Dr. Alexander H. Smith, Univ. of Mich., with the following observation: "The disagreeable taste, copious mycelium around the stipe and in the surrounding duff and more or less leather-colored cap are distinctive."

In the duff at base of stumps of *Pinus ponderosa* Laws. cut 2 years earlier, Defiance Plateau, Navajo Indian Reservation, Ariz., Sept. 19, 1957, *S. R. Andrews and W. E. Eslyn.*

Singer and Smith (3) indicate that the species is known from Wash. and Calif. This is apparently the first report of its occurrence in Ariz.

ON PSEUDOTSUGA MENZIESII (MIRB.) FRANCO

1. *Cytospora kunzei* Sacc. var. *kunzei*

Spores 3.5–5.3 × 1.0 μ; sporophores 14–18 μ long, branched and verticillately arranged at base.

On live branches of Douglas-fir, 4 miles south of Luna, N. Mex., Apache National Forest, January 29, 1959, *David McComb.*

Apparently the first report of this fungus on Douglas-fir in N. Mex.

ON PINUS EDULIS ENGELM.

1. *Sclerophoma* sp.

Spores hyaline, pointed slightly at one end, 6.3–8.1(–9.5) × 3.2–4.2 μ; no conidiophores apparent; pycnidia innate-erumpent, nonostiolate, 158–180 μ high × 105–150 μ wide.

Compared with specimens of *S. pithyophila* (Cda.) v. Höhnel in the National Fungus Collections, with which it is similar. The spores of the present specimen are much broader however.

On needles of pinyon pine, Manzano Mts., Cibola National Forest, N. Mex., Dec. 18, 1958, *W. E. Eslyn.*

ON OPUNTIA SP. (CHOLLA)

1. *Teichospora muricata* Ell. & Ev.

Perithecia erumpent, collabent, papillate, black, about 280–480 μ diam; asci 90–122 × 10.8–14.4 μ; paraphyses numerous; spores brown, muriform, 3-septate plus usually two longitudinal septa, 15–25 × 7.2–10.8 μ.

On skeletal remains of cholla, near Drake, Ariz., Sept. 1958, *W. E. Eslyn.*

Apparently a new host record for this fungus.

ON QUERCUS GAMBELII NUTT.

1. *Valsa ambiens* Pers. ex Fr.

Stromata numerous, piercing the epidermis by means of small, white discs; ostiola black, erumpent about the periphery of the disc, less often, scattered over the disc; asci 40–67 × 8.1–14.1 μ, subsessile to stalked, 4- to 8-spored; spores hyaline, curved, 14.1–24.5(–35) × 3.5–6.0(–7.1) μ.

On dead leader of oak sapling, Whitetail Unit, Mescalero Apache Reservation, N. Mex., June 4, 1959, *S. R. Andrews and W. E. Eslyn.*

Not previously reported on this host.

ON YUCCA SP.[2]

1. *Phaeodothis yuccae* (Ell. & Ev.) Sacc.

Stroma amphigenous, erumpent, thickly scattered, black; perithecial locules about 62–88 × 45–62 μ; asci oblong-cylindrical, 54–78 × 12–15.2 μ, expanding to 134 μ in length in distilled water; spores brown, 1-septate (in one case 2-septate), constricted at the septum, 14.4–16.6 × 7.1–8.1 μ.

On the lower discolored or dead leaves of *Yucca* sp. (the specimen collected was insufficient for definite diagnosis of the host; however, Dr. Dittmer noted that it was probably *Y. baileyi* Woot. & Standl.), Defiance Plateau, Navajo Indian Reservation, N. Mex., Sept. 18, 1957, *S. R. Andrews & W. E. Eslyn.*

Previously reported from Colo. on *Y. glauca.* Apparently a new host and regional record for this fungus.

ON YUCCA GLAUCA NUTT.

1. *Coniothyrium concentricum* (Desm.) Sacc.

Pycnidia arranged concentrically within a raised area of the leaf epidermis, or scattered, erumpent, spores forming a black matrix within; spores 3.5–7.1 × 3.8–7.1 μ (4–5 × 3–4 μ, Sacc.), globose to oval, mostly the former, smooth.

Cf. Herbarium University of California No. 411907, courtesy of Dr. Lee Bonar. The spores in this collection were found to measure 3.9–6.4 × 3.9–7.1 μ.

[2] The *Yucca* species were identified by Dr. Howard J. Dittmer, Department of Biology, University of New Mexico, Albuquerque, N. Mex.

On dead leaves of *Y. glauca*, near Magdalena, N. Mex., Oct. 29, 1958, *W. E. Eslyn*. *C. concentricum* var. *yuccae-glaucae* Sacc. was also found on this host near Glenwood, N. Mex., but has been reported previously on *Y. glauca* from this State (2).

ON YUCCA ELATA ENGELM.

1. *Kellermania yuccaegena* Ell. & Ev.

Pycnidia black, densely scattered, amphigenous, innate-erumpent, subglobose, 300–345 μ wide × 270–345 μ high; appearing first as brown spots with black borders and finally as black spots on the periderm of the host; pycnidial initials consisting of a completely immersed prosenchymatous mass that invades and completely fills the epidermal cells above the forming pycnidium, appearing somewhat clypeate-like; the mature pycnidial wall consisting of a lighter colored, thin inner peridium surrounded on 3 sides by a pseudoparenchymatous to prosenchymatous dark brown wall that is up to 53 μ in thickness, covered at the apex by a wall of tightly appressed parallel hyphae whose upward extension apparently splits the epidermal host cells, pierced at the center by an ostiole approximately 50 μ in width; spores hyaline, 1- to 3-celled, mostly 2-celled, granulate, cylindrical, constricted slightly at the septum, 49–104 × 10.8–14.4 μ containing an appendage at the apex 11–32 μ in length; sporophores appearing as a part of the peridial cells.

On discolored and dead leaves of *Y. elata*, near Mountainair, N. Mex., March 14, 1958, *W. E. Eslyn*.

K. yuccaegena has been collected by the author on *Y. glauca* at both Glenwood and Magdalena, N. Mex. The spores measured respectively, 43–64 × 10.6–14.1 μ and 45–60 × 9.9–10.6 μ. The herbarium specimen of this fungus examined (F. P. 14145) contained spores ranging from 35–50 μ long. The characteristics of *K. yuccaegena* on *Y. glauca* and those of the *Kellermania* as described above on *Y. elata* were the same except for the greater spore length of the latter. On the basis of these spore differences, it is believed that the isolation described may constitute a new variety of *K. yuccaegena*. However, before arriving at this conclusion definitely it would be necessary to conduct cross-inoculations to determine whether the spore differences result from host reactions.

ON GYMNOSPORANGIUM JUVENESCENS KERN ON JUNIPERUS SCOPULORUM SARG.

1. *Cladosporium herbarum* Pers. ex Link

Spores brown, 1–3-celled, 5.4–7.2 × 10.8–18.0 μ; sporophores brown, branching, 91–113 μ long.

On deteriorating telial galls of *G. juvenescens,* Jemez Canyon, N. Mex., Sept. 5, 1957, *S. R. Andrews and W. E. Eslyn.*

Apparently a new host record for this fungus.

SUMMARY

A total of 18 different fungi, which are believed to be new host or regional records for Arizona or New Mexico, have been collected on forest trees or forest-associated hosts.

ACKNOWLEDGMENTS

The author is indebted to Drs. J. C. Gilman and L. H. Tiffany, Department of Botany and Plant Pathology, Iowa State Univ. of Sci. & Tech., Ames, Iowa, for aid in identifying some of the fungi, and to Mr. John Stevenson, Prin. Mycologist in Charge, National Fungus Collections, Plant Industry Station, Beltsville, Md., for use of certain collections of the U. S. Department of Agriculture.

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STUDIES OF CLONAL ISOLATES OF ERYSIPHE CICHORACEARUM ON LEAF DISK CULTURE [1, 2]

RALPH M. MORRISON

(WITH 6 FIGURES)

INTRODUCTION

The report by Yarwood (**1935**) of the heterothallic nature of *Erysiphe cichoracearum* DC. ex Merat is based on experimental data from a study of 2 compatible single conidium cultures. Allen (**1936**) assumed the presence of 2 mating types for *Erysiphe cichoracearum* and *E. Polygoni* DC., but she presented no data. I have attempted to resolve the origin of the ascocarp by using data from the mating reactions of conidial clones maintained upon leaf disks.

METHODS

Leaf Disk Culture. Leaves were removed from the uppermost nodes of uninfected *Helianthus annuus* L. and *Zinnia elegans* Jacq. Disks were cut from the leaves with a 1.6 mm diam cork borer. The disks were immediately transferred to a culture chamber consisting of 100 ml Petri dish, a glass-rod triangle, a porous disk of 9 cm filter paper, and 25 to 30 ml of medium. The medium used in most cases was distilled water. Host tissues of both species of plants would maintain themselves and the mildew for a period long enough to allow *E. cichoracearum* to complete its cycle from conidium to ascocarp. Richmond and Lang (**1957**) reported that kinetin (6-furfurylamino purine) extended the longevity of detached *Xanthium* leaves. I tested several concentrations of this and found that 0.4 ppm extended the lifetime of the infected leaf disks of both species of host plants. Yarwood's method

[1] This paper represents a portion of a thesis presented to the Graduate School of Indiana University in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

[2] The author wishes to express his appreciation to Dr. R. M. Johns for his valuable help in the preparation of this paper, to Dr. C. O. Miller for supplying the kinetin used in the leaf disk experiments, and to Irena Morrison for her help in preparing the photographs.

(1935) with sucrose was tried as a medium, but contaminations were too frequent, and the method was abandoned. Yarwood (1946) and Schnathorst (1958) suggest 18 to 20° C for maintenance of mildew upon detached leaves. The optimum for *E. cichoracearum* on *H. annuus* and *Z. elegans* tissue was 18° C. For illumination, a bank of six, 40 watt, G. E., cool-white fluorescent lamps was attached to a movable fixture. Schnathorst (1958) reports that 300 ft-c is optimum for the maintenance of detached *Lactuca* leaves in culture with *E. cichoracearum*. Several intensities were tested, but 300 ft-c was used as the optimum, as the disk-mildew cultures lasted longer, and the yield of conidia was appreciably greater. The photo-period consisted of 18 hr light and 6 hr darkness.

Clonal Cultures. Single spore cultures of *E. cichoracearum* were obtained by dusting conidia onto 1% agar; single conidia were picked from the agar with a fine needle. Single spores, isolated from a specific host, were inoculated upon uninfected tissue of the same host plant. About 16% of the spores inoculated produced infection and viable clones. This method permits observation, with a binocular microscope, of germination and subsequent growth and development.

RESULTS

At 18° C, mildew colonies appeared 7–10 days after inoculation of the leaf disk. The leaf disk became chlorotic as the mildew colony

TABLE I

CONFRONTATIONS OF CLONES OF ERYSIPHE CICHORACEARUM ISOLATED FROM HELIANTHUS ANNUUS

ha	17	21	23	10	11	18	19	20	22	24	25	26	27
10	+	+	+	−	−	−	−	−	−	−	−	−	−
11	+	+	+	−	−	−	−	−	−	−	−	−	−
18	+	+	+	−	−	−	−	−	−	−	−	−	−
19	+	+	+	−	−	−	−	−	−	−	−	−	−
20	(−)	+	+	−	−	−	−	−	−	−	−	−	−
22	+	+	+	−	−	−	−	−	−	−	−	−	−
24	+	+	+	−	−	−	−	−	−	−	−	−	−
25	+	+	+	−	−	−	−	−	−	−	−	−	−
26	+	+	+	−	−	−	−	−	−	−	−	−	−
27	+	+	+	−	−	−	−	−	−	−	−	−	−
17	−	−	−	+	+	+	+	(−)	+	+	+	+	+
21	−	−	−	+	+	+	+	+	+	+	+	+	+
23	−	−	−	+	+	+	+	+	+	+	+	+	+

Clones 10 and 11 were isolated 9/58 from *H. annuus* in the botanical experimental field. Clones 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, and 27 were isolated 11/58 from *H. annuus* in the greenhouse. (−) no cleistothecia formed after repeated matings. +cleistothecia. −no cleistothecia. Crosses made 3/59.

TABLE 2

CONFRONTATIONS OF CLONES OF ERYSIPHE CICHORACEARUM ISOLATED FROM HELIANTHUS ANNUUS AND ZINNIA ELEGANS

ze/ha	17	21	23	10	11	18	19	20	22	24	25	26	27
1	+	+	+	−	−	−	−	−	−	−	−	−	−
2	+	(−)	+	−	−	−	−	−	−	−	−	−	−
4	+	+	+	−	−	−	−	−	−	−	−	−	−
6	+	+	+	−	−	−	−	−	−	−	−	−	−
7	+	+	+	−	−	−	−	−	−	−	−	−	−
26	+	+	+	−	−	−	−	−	−	−	−	−	−
28	(−)	(−)	+	−	−	−	−	−	−	−	−	−	−
5	−	−	−	+	+	+	+	+	+	+	+	+	+
16	−	−	−	+	+	+	+	+	+	+	+	+	+
27	−	−	−	+	+	+	+	+	+	+	+	+	+
29	−	−	−	+	+	+	+	+	+	+	+	+	+
10	−	−	−	−	−	−	+	−	−	−	−	−	+
12	−	−	−	−	−	−	+	−	−	−	−	−	+
20	−	−	−	−	−	−	+	−	−	−	−	−	+
3	−	−	−	−	−	−	−	−	−	−	−	−	−
22	−	−	−	−	−	−	−	−	−	−	−	−	−

Clones ze (*Z. elegans*) 1, 2, 3, 4, 5, 6, 7, and 10 were isolated 7/58 from *Z. elegans* in the experimental field. Clones 12, 16, 20, 22, 26, 27, 28, and 29 were isolated 8/58 from *Z. elegans* in the experimental field. ha *H. annuus*. Crosses made 10/58.

advanced. Sporulation became more extensive, until disk was covered with conidial chains. *E. cichoracearum* will sporulate upon *H. annuus* disks for 5 to 6 weeks, and on *Z. elegans* for 7 to 8 weeks. After these periods it was necessary to transfer the clones to fresh disks to insure their continued growth. A solution of 0.4 ppm kinetin would keep the leaf disk-mildew cultures going for longer periods (7 weeks for *H. annuus* and 10 weeks for *Z. elegans*).

TABLE 3

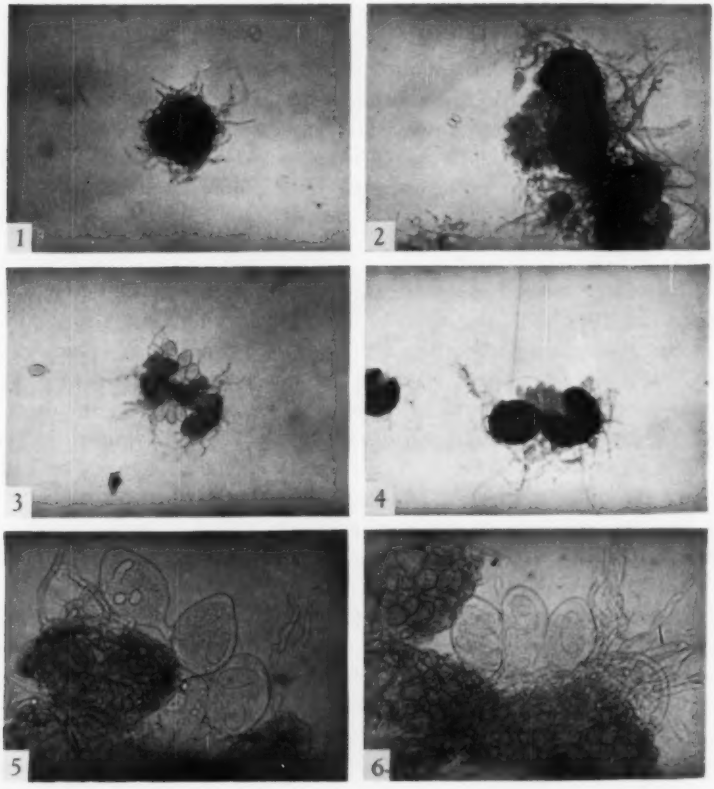
CONFRONTATIONS OF CLONES OF ERYSIPHE CICHORACEARUM ISOLATED FROM ZINNIA ELEGANS

ze	1	2	4	6	7	26	28	5	16	27	29
5	+	+	+	+	+	+	+	−	−	−	−
16	+	+	+	+	+	+	+	−	−	−	−
27	+	+	+	+	+	+	+	−	−	−	−
29	+	+	+	+	+	+	+	−	−	−	−
1	−	−	−	−	−	−	−	+	+	+	+
2	−	−	−	−	−	−	−	+	+	+	+
4	−	−	−	−	−	−	−	+	+	+	+
6	−	−	−	−	−	−	−	+	+	+	+
7	−	−	−	−	−	−	−	+	+	+	+
26	−	−	−	−	−	−	−	+	+	+	+
28	−	−	−	−	−	−	−	+	+	+	+

Clones 1, 2, 4, 5, 6, and 7 were isolated 7/58 from *Z. elegans* in the experimental field. Clones 16, 26, 27, 28, and 29 were isolated 8/58 from *Z. elegans* in the experimental field. Crosses made 9/59.

Once clonal isolates were in culture, experiments were designed to determine whether cultural conditions would favor ascocarp development upon leaf disks, provided the potential for such a structure was present within a strain or any compatible combination of strains.

A total of 36 clones have been isolated from *H. annuus* (13 clones) and *Z. elegans* (23 clones) and maintained upon leaf disks for 12 months. All 36 were inoculated onto the 2 species of host tissue. Both species of host tissue were divided into 2, one set sustained by distilled water and another by a 0.4 ppm kinetin solution. Each clone was inoculated onto 4 disks per each medium, thus 288 infections were obtained. At



FIGS. 1–6. *Erysiphe cichoraccarum*. 1, 3, 5. Cleistothecium collected from leaf disk culture (cross ha 17 × xe 7). 2, 4, 6. Cleistothecia collected from *Helianthus annuus* growing in the greenhouse. 1–4. Magnified 95 ×. 5, 6. Magnified 265 ×.

intervals, the colonies were examined for cleistothecia. During the 8 weeks of the experiment, none appeared.

Matings were then performed by transferring conidial masses from clonal cultures to fresh leaf disk tissue. *H. annuus* and *Z. elegans* served as substrata and each clonal mating was done in quadruplicate. Both distilled water and a 0.4 ppm kinetin solution were used as media for the leaf tissues. The matings were as follows: 13 *H. annuus* clones × themselves (TABLE 1); 13 *H. annuus* clones × 16 *Z. elegans* clones (TABLE 2); 11 *Z. elegans* clones × themselves (TABLE 3).

Cleistothecia appeared in 3 to 4 weeks as yellow to yellow-brown knots of mycelium. By 5 weeks the ascocarps were black and macroscopically visible. The ascocarps were usually concentrated at or near the inoculation site of the 2 compatible clones. Ascocarps formed on disks on both the distilled water and the kinetin solution. These data suggest the presence of 2 mating types for *E. cichoracearum*. These types have been designated as "plus" and "minus." The cleistothecia produced by compatible clones (FIGS. 1, 3, 5) were morphologically indistinguishable from those collected from plants in nature (FIGS. 2, 4, 6).

DISCUSSION

My culture method has eliminated difficulties met by earlier workers (Yarwood, **1946**). Using disks exposes a greater area of tissue to the medium, than does placing the petiole into the medium. Placing the tissue on moist filter paper stabilizes the disks and permits greater ease of handling. The medium is simple. The mildew will complete its cycle on disks maintained upon distilled water. The *Zinnia* disks tend to form adventitious roots on distilled water. Their longevity and support of the mildew were greater than disks that formed no roots.

The data substantiate Yarwood's (**1935**) work. *E. cichoracearum* is heterothallic, and data from matings indicate 2 mating types, designated as "plus" and "minus."

Several matings were negative (TABLE 2). The clones in question have been mated several times with no ascocarps being formed. *Zinnia* clones 10, 12, and 20, cross with *Helianthus* clones 19 and 27, but not with *Helianthus* 10, 11, 18, 20, 22, 24, 25, and 26. *Zinnia* clones 3 and 22 do not cross with any of the *Helianthus* clones. These data suggest that additional factors determine compatibility in several clonal strains.

SUMMARY

Leaf disks maintained on distilled water or with the addition of 0.4 ppm of kinetin have proved effective for the culture of *Erysiphe cichora-*

cearum. Thirty-six clonal isolates of *E. cichoracearum* from *Helianthus* and *Zinnia*, which were grown by this method, failed to produce cleistothecia. However, intercrosses between these clones resulted in cleistothecia after 3 to 4 weeks. The crossing data confirm heterothallism in *E. cichoracearum* and establish the presence of 2 mating types. With the exception of a few specific crosses, isolates from *Zinnia* are interfertile with those from *Helianthus*. These exceptions suggest that additional factors affect compatibility in certain strains.

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PLEOMORPHISM AND GROWTH CYCLES IN TRICHOPHYTON MENTAGROPHYTES

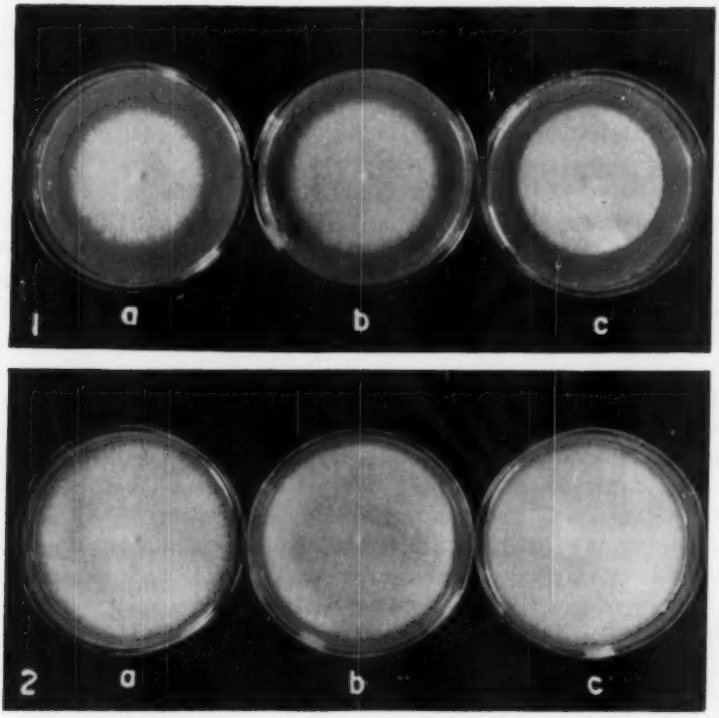
G. N. Bistis

(WITH 7 FIGURES)

Pleomorphism is the term applied to a type of cultural instability often encountered in laboratory culture of most species of dermatophytes. It is manifested by the appearance, at scattered points over the surface of a colony, of tufts of fluffy, white aerial hyphae (FIG. 3) which spread rapidly and eventually blanket most of the original aerial mycelium. When mass transfers are made from individual tufts to fresh medium, the resultant colonies are morphologically very different from the parent colony. In general, they are faster growing, less pigmented and produce fewer conidia. These variant colony types are termed "pleomorphic" and the strain from which they arose is usually referred to as the conidial or normal form (FIGS. 1a, 2a).

The search for the causes of and the steps in the phenomenon of pleomorphism in these fungi, although extensive, has not been very fruitful. In a recent short review article on pleomorphism in *Trichophyton mentagrophytes* (Robin) Blanchard (Bistis, **1959**) the results of 60 years of inquiry by various workers have been correlated with my own observations and forged into a simple testable hypothesis summarized briefly as follows.

In the life history of a conidial colony of this species, mutations of various sorts would be expected to occur in a small proportion of the cells (Emmons, **1932**). The detection of the pleomorphic mutations is made possible by two factors: (1) the mutant cells have the ability to proliferate in areas previously occupied by the established mycelium; (2) the aerial hyphae of the mutant grow above and over the original aerial mycelium. Each tuft of aerial mycelium, plus the underlying surface and subsurface hyphae, represents a colony, the mutant, growing within a colony, the established conidial type. Mass transfer from a tuft gives rise to a genetically homogeneous colony of a particular morphology. However, the colonies from different tufts differ in conidium production and also in other characters such as pigmentation, rate of growth, etc. A whole spectrum of types is found (Cantanei, **1929**; Robbins and McVeigh, **1949**) ranging from those which closely resemble



FIGS. 1, 2. 14-day-old colonies of three strains of *Trichophyton mentagrophytes* on neopeptone-dextrose medium. 1. 25°. 2. 35°. a. Conidial strain (*N*). b. Intermediate strain (6–1). c. Highly pleomorphic strain (III).

the parent strain, to the highly pleomorphic type which produces few or no conidia. Moreover, the original conidial strain can mutate directly to one of the intermediate forms (FIG. 1b, 2b) or to a highly pleomorphic type (FIG. 1c, 2c) (Bistis, **1959**).

As with other asexual organisms, the term mutation is used here in the sense of a heritable change. The identity of the altered unit is at present unknown.

The hypothesis suggests that the eruption of a pleomorphic tuft over the surface of a colony involves several processes, with initiation and expression being the two end points. That one of the steps in the expression of the mutation as a visible tuft of mycelium is a growth phase has been clearly demonstrated (Bistis, **1959**). Each tuft represents a clone or colony of mutant cells which attain visible proportions

mainly because of an ability to grow within the meshes of an established conidial colony. However, once growth has been implicated as a selective factor responsible for the appearance of pleomorphic tufts, nutrition becomes a key factor. Since growth involves utilization of nutrients, it is apparent that in cultures of the conidial form, a pool of nutrients exists which are available to the pleomorphic cells.

Clues as to the identity of these nutrients are abundant in the extensive literature on the nutrition of *T. mentagrophytes*. The evidence, though indirect, points to nitrogen metabolism as the key to the puzzle. For example, Goddard (**1934**) observed that in the growth of a strain he called *Trichophyton interdigitale* Priestley, ammonia from the deamination of amino acids [1] accumulates in the medium, and that the resultant increase in pH probably results in the cessation of growth. Nickerson and Williams (**1947**) have suggested that perhaps the high concentration of ammonium ion per se, rather than pH may be the limiting growth factor. Mosher et al. (**1936**) confirmed Goddard's results and found that phosphate acted as a buffer which neutralized the growth inhibiting action of the base produced during growth. They also reported that their strain (*T. interdigitale*) was unable to grow on inorganic nitrogen but did utilize amino acids, although in a complex pattern. A pattern of amino acid utilization, somewhat different from that reported by Mosher et al., was found by Robbins and Ma (**1945**) for a normal strain of *T. mentagrophytes*. They also made the very significant discovery that some pleomorphic variants isolated from this normal strain could grow with NH_4NO_3 as a sole source of nitrogen, whereas this compound was totally unavailable to the normal form as a nitrogen source. In subsequent papers (Robbins, **1947**, **1950**), it was suggested that the presence of new or more efficient enzyme systems in the pleomorphic forms may be responsible for the greater efficiency in their nitrogen metabolism. This gain in growth potential was further investigated by McVeigh and Cambell (**1950**) who grew normal and pleomorphic strains on a number of single amino acids. They found that the pleomorphic forms grew better (higher dry weights) on a number of different amino acids. Lastly, Fujii (**1957**) confirmed these reports of ammonium utilization and more efficient utilization of amino acids by the pleomorphic strains.

There is, therefore, considerable evidence that in the media commonly employed, the factor limiting the growth of the conidial strain is related to nitrogen metabolism and that pleomorphism may involve an alteration

[1] Bentley (**1953**) reported the presence of enzymes for oxidative deamination of amino acids in acetone powders of mycelia of *T. mentagrophytes*.

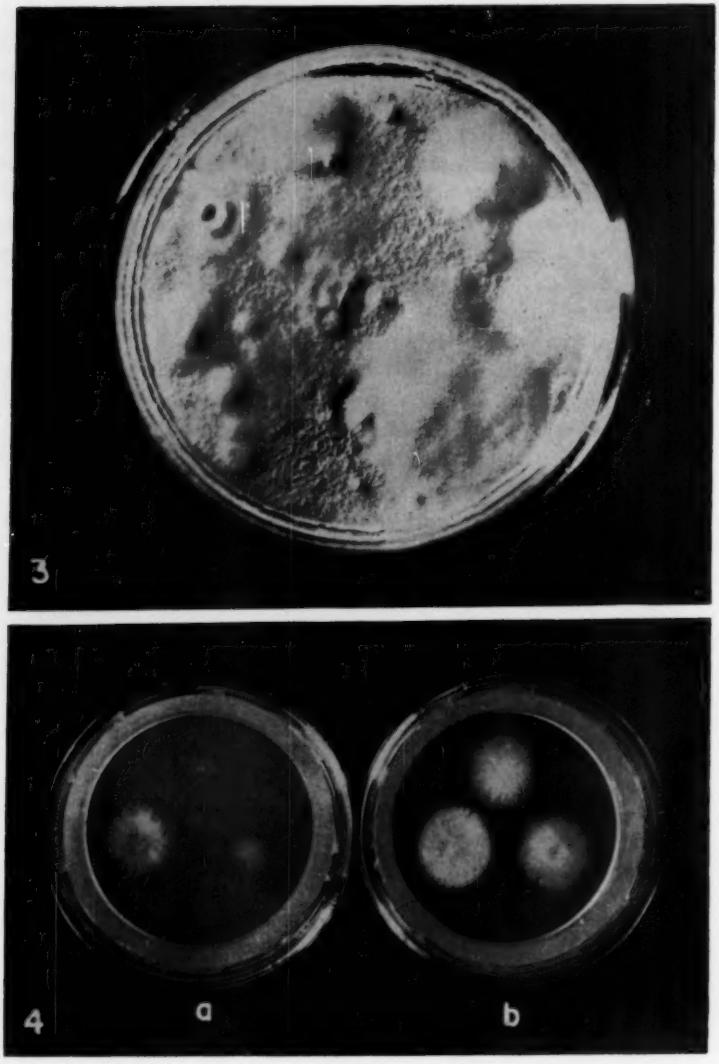
in the enzymes regulating nitrogen utilization. The growth phase of the conidial strain would end presumably when one of the following conditions was reached: (1) increase in pH due to ammonia accumulation; (2) increase of the ammonium ion concentration to toxic levels; (3) exhaustion of nitrogen sources available to the normal form. During the growth phase, however, mutations are almost certainly occurring and selection will favor a mutant which can overcome the effective limiting factor. The pleomorphic variants appear to be such mutants. Perhaps they grow over a wider pH range, but no data on comparative pH tolerance are available. If the second factor is the effective one, their ability to utilize ammonia indicates that they may be less sensitive to ammonia inhibition than the normal form. But even if sensitivity is unaltered, their ability to utilize ammonia may remove enough of the ion, at least locally, to minimize its toxic action. If the limiting factor is the third alternative, the pleomorphic variants again have the selective advantage, both in their ability to utilize ammonia and in their more effective utilization of amino acids.

In all of the previous work, however, growth was measured either visually or as dry weight attained after a given period of growth. The limitations of these methods are too well known to enumerate here. For a more accurate picture of the differences in growth potential among the various strains, a study of the kinetics of growth was required. The growth curves presented in this paper for the normal, an intermediate, and a highly pleomorphic strain represent a short first step in the study of growth kinetics in this species. An analysis of the growth curves will be the subject of the latter section of this paper. The first section will deal with a simple experiment which demonstrates visually the ability of pleomorphic cells to proliferate in stationary-phase conidial cultures.

MATERIALS AND METHODS

Of the three strains of *Trichophyton mentagrophytes* used in these experiments, the one designated *N* is the conidial, "normal" strain of Robbins and Ma (**1945**). The other two are pleomorphic variants of the *N* strain isolated by mass transfer from two patches which appeared on an ageing *N* culture. Since both cultures derived from the mass transfers produced some microconidia, two homokaryotic strains were established by single microconidial transfer. One strain, an intermediate (FIG. 1b, 2b) was designated 6–1; the other, a highly mycelial strain (FIG. 1c, 2c) was designated III.

Stock cultures of the three strains were maintained on neopeptone-



FIGS. 3–4.

FIGS. 3, 4. 3. Pleomorphic patches overgrowing a 28-day-old *N* mycelium incubated at 35° on neopeptone-dextrose medium. 4. The ability of the pleomorphic variants to grow upon an established *N* mycelium as illustrated by the double-dish method of cultivation. Cultures grown on neopeptone-dextrose medium

dextrose agar slants in a 25°C incubator and transferred at weekly intervals.

The two media used in the growth experiments were as follows:

Neopeptone-dextrose medium per 1000 ml		Casein-dextrose medium per 1000 ml	
A.			
KH_2PO_4	1.5 g	KH_2PO_4	1.5 g
$MgSO_4 \cdot 7H_2O$	0.5 g	$MgSO_4 \cdot 7H_2O$	0.5 g
Neopeptone	2.0 g	Casein hydrolysate[2]	2.0 g
Dextrose	50.0 g	Dextrose	50.0 g
Thiamine	0.5 mg	Mineral supp.[2]	0.5 ml
Mineral supp.[2]	0.5 ml	Washed agar[2]	15.0 g
Bacto agar	15.0 g		
B.			
KH_2PO_4	10.5 g		

[2] See Robbins and Ma (1945).

In all growth experiments, the fungus was cultured in 9 cm petri dishes containing 30 ml of agar medium. The neopeptone-dextrose agar was prepared in two double strength fractions (A & B) which were distributed into test tubes (15 ml per tube) and autoclaved at 15 lbs for 15 min. After sterilization, the contents of two tubes (one A and one B) were poured into a 9 cm petri dish.

The harvesting of the mycelia was facilitated by placing a physical barrier (a cellulose membrane) between the mycelium and the agar medium by means of a double-dish method previously described (Bistis, 1957). The inner dish was prepared as follows: A moistened disk of cellulose membrane (10 cm in diam) was balanced on the rim of a 9 cm petri dish containing 10 ml of distilled water. The membrane was forced down into the dish with an 8 cm ring of leucite. In this way, an "all membrane" dish was fashioned with an 8 cm base and a lateral wall 1 cm high. The leucite ring was then removed, the lid of the petri dish replaced and the entire assembly autoclaved. After sterilization, the cooled cellulose membrane "inner dish" was removed with sterile forceps and set directly upon the surface of the agar layer in a petri dish, the "outer dish."

at 35°. a. Differential growth achieved by three strains in 5 days on a cellulose membrane placed over an *N* mycelium (4–9th day). Upper: sparse growth of the conidial strain (*N*). Lower right: somewhat more extensive growth of the intermediate strain (6–1). Lower left: vigorous growth of the highly pleomorphic strain (III). b. Growth of the same three strains on a control plate (no mycelium below the membrane).

Plates were routinely inoculated with a suspension of microconidia obtained from 7–10-day-old cultures incubated at 25°. The aerial mycelium bearing the conidia was scraped off, shaken in 10 ml of H_2O and passed through a milk filter disk. The concentration of the filtrate was adjusted to 10^6 microconidia/ml and a 1 ml sample of the suspension spread uniformly over the membrane in each plate. In one instance (III curve, FIG. 5), the inoculum consisted of 5.6×10^3 mycelial fragments.

There were many variations in the methods of harvesting and weighing the mycelial mats. In general, the mats were dislodged from the membrane with a single-edge razor, washed with distilled water onto Whatman #2 filter paper in a Buchner funnel, and dried at 95–105°.

In some of the long-range experiments the agar tended to dry out at the high temperature of incubation (35°). This problem was overcome by stacking the plates in tall glass cylinders to which some water had been added. A glass plate placed asymmetrically over the top of each cylinder reduced evaporation but still allowed for gas exchange.

RESULTS

Growth Potential.—The following procedure is a rather simple but effective method of demonstrating the ability of pleomorphic hyphae to grow in stationary-phase conidial cultures. Two plates of neopeptone-dextrose agar were prepared and one inoculated over the entire surface with a suspension of *N* microconidia. Nothing was added to the second one. Both plates were incubated at 35° for four days, at which time the *N* culture in the inoculated plate was entering the stationary phase of the growth cycle (FIG. 5). The aerial mycelium was then scraped from the colony and a leucite ring-cellulose membrane dish (Bistis, **1957**) was set directly on the surface of each plate. Agar blocks bearing single hyphal tips of each of the three strains were placed on the membrane surfaces of the two plates. The dishes were then incubated for five or more days at 35°. In the plate with the submembrane *N* mycelium (FIG. 4a) there was a marked difference in the amount of growth achieved by the three strains. The *N* strain grew very sparsely; the 6–1 strain (an intermediate) grew somewhat better, while III (a highly pleomorphic isolate) grew almost as well as on the fresh agar plate. In this second plate (FIG. 4b) growth of all three isolates was quite vigorous with only very minor differences in the amount of growth. This simple experiment graphically demonstrates the ability of pleomorphic cells to proliferate in areas already occupied by an established *N* mycelium. It

further indicates that this ability varies among the different pleomorphic strains and that the differences in growth potential may be correlated with differences in conidium production. The strains can be arranged in the following series: $N \rightarrow 6\text{–}1 \rightarrow$ III according to increase in growth potential and reduction in number of conidia.

The lack of agreement between these results and those of Robbins and Ma (**1945**) may be due to the difference in experimental procedure.

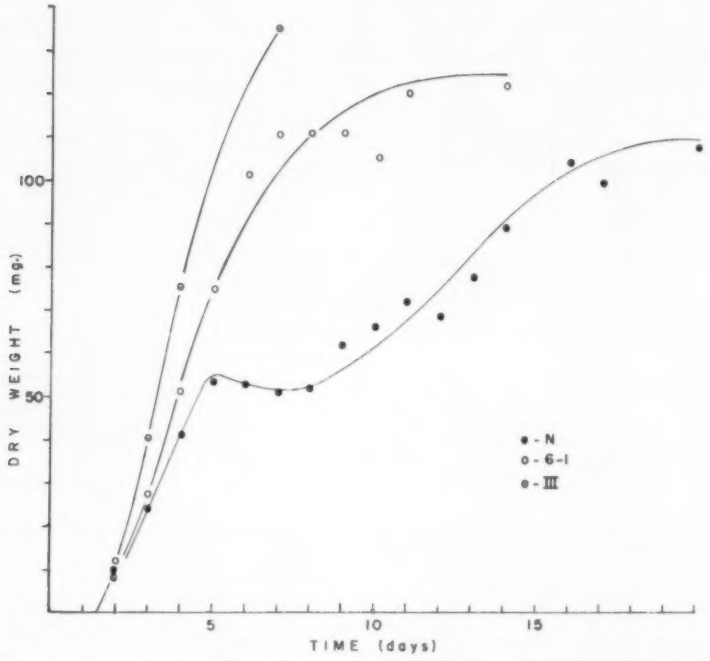


FIG. 5. Growth curves for the three strains grown on neopeptone-dextrose medium and incubated at 35°.

They reported that cell-free extracts from various strains when added to fresh medium, benefited the growth of that particular strain and all less highly pleomorphic strains. Their procedure, however, was such that any volatile or unstable metabolic products would be lost from the extract.

Growth Curves.—Although the experiment described above illustrates graphically the difference in metabolic capacity among the three strains, it involves the interaction of two cultures growing together and

would thus be difficult to analyze further. A quantitative comparison of growth capacity in single-strain culture, however, would be a less involved way of studying the differences observed. Consequently, a number of double-dish agar cultures were set up and dry weights taken at regular intervals.

The points on the curves are averages of varying numbers of replicates and some, therefore, represent more accurate averages than others. The complete data are presented only for strains *N* and III on the casein hydrolysate-dextrose medium at the critical times of 72 and 102 hours after inoculation (TABLE II). These are the only data which were analyzed statistically (the t-test for significance of the differences of means).

In a neopeptone-dextrose medium (FIG. 5) the growth cycles of the three strains differ markedly; the results demonstrate that a positive

TABLE I

MEAN DRY WEIGHTS* (MG/30 CC MEDIUM)
GROWTH OF THREE STRAINS OF T. MENTAGROPHYTES ON CASEIN HYDROLYSATE-DEXTROSE MEDIUM

Strain	Time (hours)							
	48	54	72	78	96	102	120	126
N	8.3	15.9	36.6	41.4	53.4	59.6	60.9	70.3
6–1	7.9	13.7	35.9	43.8	56.9	62.6	76.9	89.1
III	5.1	11.7	34.1	47.6	82.4	91.1	123.8	121.3

* The means for 72, 78, 96, and 102 hours were calculated from five or six replicates. The others are averages of three replicates.

correlation exists between growth capacity and the level of the pleomorphic transformation. This is reflected by an apparent increase in growth rate, a lengthening of the growth period, and an increase in final dry weight as the expression of the pleomorphic phenotype intensifies.

A complicating factor, however, is the resumption of growth in the *N* cultures after the eighth day. Although this two-lag-phase, two-growth-phase cycle resembles superficially the diauxie phenomenon (Monod **1942**, **1949**) encountered in a number of bacteria, there is a fundamental distinction between these two examples of two-phase growth. The resumption of growth observed by Monod was due to the synthesis of new enzyme systems in cells of unaltered genotype, whereas in the conidial form of *T. mentagrophytes*, the second phase is apparently due to the proliferation of the pleomorphic variants, since

this is the time (8–12th day) when pleomorphic tufts are erupting over the surface of the *N* culture. A change in genotype, which may possibly introduce new enzyme systems, is responsible for the second growth phase in the normal form of *T. mentagrophytes*.

If nitrogen metabolism, as suggested in the introduction, is the key to the phenomenon of pleomorphism, the unraveling of the relationship requires a study of the kinetics of growth on simpler nitrogen sources.

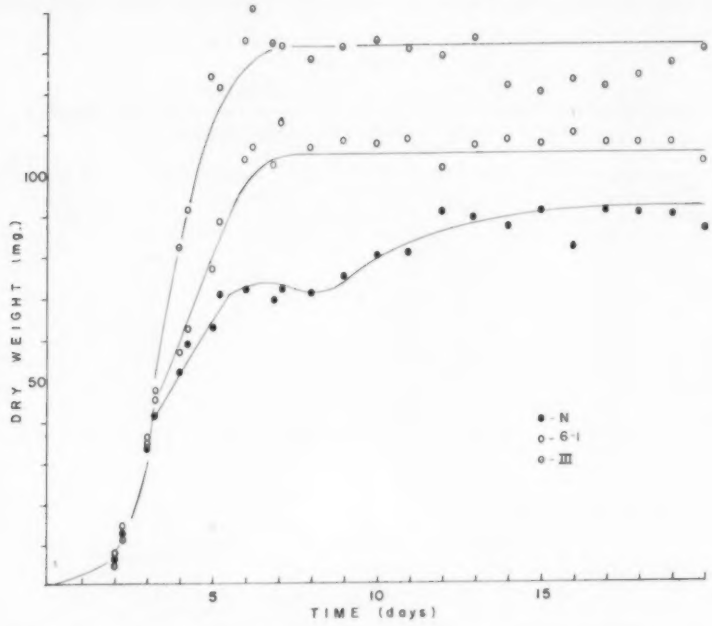


FIG. 6. Growth curves for the three strains grown on casein-hydrolysate medium and incubated at 35°.

As a step toward this goal, the three strains were cultured on a casein hydrolysate-dextrose medium, weights determined (TABLE I), and growth curves constructed (FIG. 6).

Again the differences in final dry weight are quite apparent, but the other differences are not present or have been modified. All three strains grow at the same rate for the first 72 hrs (TABLE II) ($t = 1.47$, $p = 0.2$), but then all suffer a sharp reduction in growth rate. It appears that the severity of the drop is correlated with the degree of pleo-

morphism. The sharpest break occurs in the conidial (N) cultures; the least decline in the highly pleomorphic (III) strain. The second growth phase ceases about the same time (ca. 6th day) in all cultures, and the differences in final dry weight here are due to the differences in growth rate from the third to the sixth day (TABLE II).

This type of two-phase growth with no second lag has also been reported in bacteria (Cohen, **1949**). Here the carbohydrate source was supplied as a complex rather than the nitrogen source. The bacterium, *Escherichia coli,* was grown in a medium containing both glucose and

TABLE II

DRY WEIGHT (MG/30 CC MEDIUM)
GROWTH OF THE NORMAL STRAIN (N) AND A HIGHLY PLEOMORPHIC STRAIN (III) OF T. MENTAGROPHYTES ON CASEIN HYDROLYSATE-DEXTROSE MEDIUM

Strain	Time 72 hrs		Time 102 hrs	
N	35.9		64.2	
	38.1		53.5	
	35.5	$\bar{x} = 36.6$	60.1	$\bar{x} = 59.6$
	40.5		54.5	
	33.4		56.1	
	36.0		69.3	
III	35.6		92.1	
	32.1		93.3	
	33.8	$\bar{Y} = 34.1$	84.5	$\bar{Y} = 91.1$
	29.2		90.7	
	34.5		93.6	
	39.1			

Test for significance:

$t = 1.47$ $\quad t = \sqrt{\dfrac{\bar{X} - \bar{Y}}{s^2\left(\frac{1}{N_x} + \frac{1}{N_y}\right)}} \quad t = 9.96$

$p = 0.2$ $\qquad\qquad\qquad\qquad\qquad p = 0.01$

glucuronic acid. The glucose was utilized first at a given rate (first growth phase) and then immediately upon glucose exhaustion, growth continued at a new rate (second phase) characteristic of the uronic acid. The two growth phases of *T. mentagrophytes* on casein hydrolysate can perhaps be explained in a comparable way. During the first three days of growth, the three strains are metabolizing the same nitrogen compound at the same rate, but after the third day this compound is depleted and one or several sources are now being utilized at different rates.

The growth cycle in cultures of the N strain is again complicated by the post-autolysis growth phase, although on this medium it is not as

pronounced. These relationships can be more clearly demonstrated when the weights are transformed into their cube roots (FIG. 7). The concept of a cube root growth phase was first suggested by Brown (**1922**) and then developed into a formal hypothesis by Emerson (**1950**). When the present data are so transformed, the curves approximate straight lines which show very clearly the differences in the growth rates of the various strains. A word of caution must, however, be added.

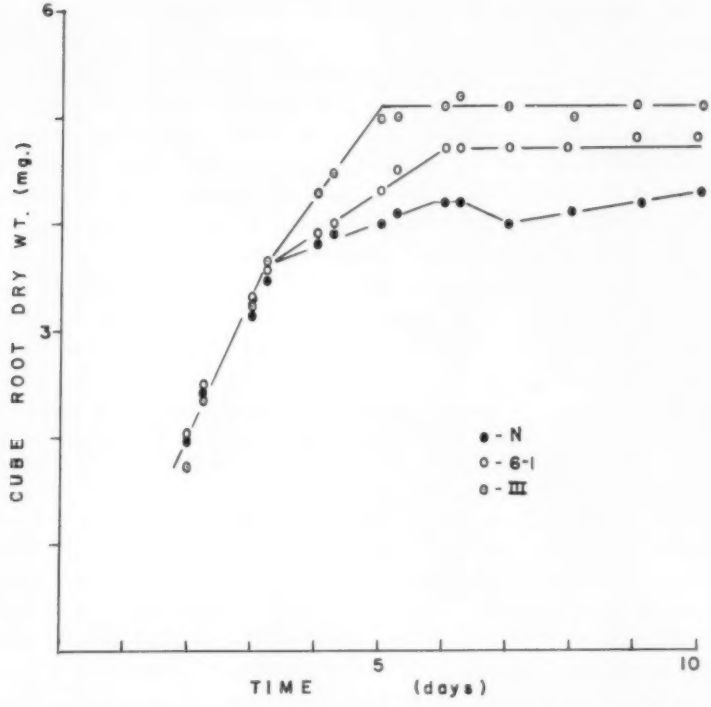


FIG. 7. Data of FIG. 6 transformed into the cube root of the dry weight.

Whether the cube root growth phase has any real meaning in a physiological sense, is still an open question, at least with respect to *T. mentagrophytes*. When the square root or even the fourth root of the dry weight is plotted, the curves will again approximate straight lines. Clearly, more extensive and exact data are required before a claim for the existence of a "cube root growth phase" in this species can be made.

DISCUSSION

There is fairly general agreement that pleomorphism in *Trichophyton mentagrophytes* is yet another example of mutation in the many-celled populations (colonies) of a microorganism. The results presented here and in a previous paper (Bistis, **1959**) demonstrate that one of the selective factors which is responsible for the expression of the pleomorphic variants is their ability to grow within the meshes of the parent cell population.

The data reported have demonstrated three different growth cycles in three strains of this species and have revealed the complexity of each cycle. The sweeping generalization that pleomorphic forms are "faster growing" than the normal is obviously an overstatement. When dry weight attained at a specific time is taken as the measure of growth, the pleomorphic forms are not always "faster growing." For example, on the casein hydrolysate-dextrose medium, the growth rates are identical during the first three days; the differences in final weights being due to differential rates during the second growth phase.

The data also reveal the inadequacy of growth measurements taken at one or two points in the growth cycle. The diauxie observed in the cultures of the normal form would give a false impression if determinations were made only at the end of the second growth phase in the neopeptone medium. Likewise, an early measurement on the casein hydrolysate medium might lead to the false conclusion of identical growth rates. The complexity of the curves requires the determination of dry weight at many points in the growth phase for an accurate picture of growth in this fungus. Since the complexity of the growth curves parallels the complexity of the nitrogen source, the hypothesis that a change in nitrogen metabolism is the nutritional basis for pleomorphism is greatly strengthened. A final proof would require the comparison of growth curves of the various strains on a less complex nitrogen source such as nitrogen-containing salts and individual amino acids. Unfortunately, previous experiments along these lines are far too inadequate to stand as final proof of a pleomorphism-nitrogen metabolism relationship. They indicate merely that the pleomorphic strains attain a higher dry weight than the normal when both are cultivated on identical media. Whether this is a reflection of a nonspecific change in phenotype which indirectly affects nitrogen metabolism, or a real gain in the ability to utilize nitrogen through new enzyme systems, remains a central problem for future investigation. The one fact we have is that under identical conditions of culture the pleomorphic forms can give a higher final dry

weight yield because of a longer growth period, a faster growth rate, or both.

Another still unexplored area is the nature of the mutated entity in the pleomorphic strains. If the different phenotypes, including the large number of intermediates, are under direct gene control, a complex genetic system must be postulated. Either several different loci, multiple alleles at one locus or perhaps pseudoalleles must be the genetic system responsible. Since each variant is probably due to a single mutation, the various alleles (or mutant loci) presumably are acting in quantitatively different ways.

The apparent negative correlation between conidium production and growth capacity—as measured by final dry weight—is still another aspect of this complex problem. The correctness of this assumption can be tested in several ways. First, a determination of final dry weight for several more intermediate strains should resolve the question. Secondly, there is considerable evidence (Davidson and Gregory, **1934**; Acton and Dey, **1934**; Chin and Knight, **1957**; Bistis, **1959**) that the intensity of conidium production, particularly in the intermediate strains (FIG. 1b, 2b), can easily be altered by changes in the environment. Growth curves obtained under some of these conditions for several strains might add the final proof for a negative correlation between conidium production and growth potential. Finally, if the basis for the differences in growth capacity resides in the pathways of nitrogen metabolism then a link between nitrogen metabolism and conidium production would be indicated. The results of such studies might have a direct bearing on similar transformations in other fungi. The transformation from conidial to mycelial growth form is not limited to dermatophytes but is exhibited by many fungi under laboratory culture. However, these examples have been studied even less than pleomorphism and are, therefore, less understood.

SUMMARY

Evidence has been presented in support of the hypothesis that the selective factor responsible for the expression of the pleomorphic variants in *Trichophyton mentagrophytes,* is their ability to proliferate in areas already occupied by the parent mycelium. The greater growth potential of the pleomorphic strains is further illustrated by growth studies with unistrain cultures. Growth curves for the conidial (*N*), an intermediate (6–1), and a highly pleomorphic (III) strain have been presented. On a neopeptone-dextrose medium, an analysis of the curves shows that

the rate of growth, final dry weight, and the length of the growth phase vary with the stage of pleomorphism reached—as measured by diminution of conidium production. The values of the growth measurements are lowest in the conidial (*N*) form and greatest in the highly pleomorphic form (III). On a casein hydrolysate-dextrose medium the differences in final dry weight are still apparent, but a different growth pattern emerges. All three strains grow at the same rate for the first three days, but then all suffer a sharp diminution in growth rate. The severity of the drop is again related to the degree of pleomorphism, the drop being greatest in the *N* and the least in the III cultures. The growth period (six days) is the same in all three strains so that the differences in final dry weight are due to the differential growth rates in the second growth phase (3rd to 6th days). In addition to the two-phase growth cycle described above, a type of growth cycle superficially resembling diauxie manifested by the conidial strain. This phase corresponds temporally with the morphological manifestation of the pleomorphic variants within the conidial colony.

ACKNOWLEDGMENTS

I wish to express my gratitude to Dr. W. J. Robbins for introducing me to the organism, the problem, and his insights into both. I also want to thank Dr. Alma Whiffen Barksdale for her interest in and encouragement of this project which was pursued during my tenure as Research Associate at the New York Botanical Garden. Finally, I express my debt to my wife, Dorothea, whose labors on behalf of the manuscript ended just before her own began.

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TAXONOMIC POSITION OF THE ECCRINALES AND RELATED FUNGI [1]

ROBERT W. LICHTWARDT

(WITH 14 FIGURES)

I doubt whether any of the seven groups of fungi under discussion in this symposium is as obscure, generally, to mycologists as are the Eccrinales and related fungi known as the Trichomycetes. The obscurity of the Trichomycetes is no fault of the fungi, except, perhaps, in that most of them have come to select the guts of living arthropods in which to grow, reproduce, and die.

The term Trichomycetes was introduced by three French workers, Duboscq, Léger, and Tuzet (**1948**) to encompass those fungi associated with arthropods, which seemed to have certain interrelationships. Included were the orders Eccrinales, Amoebidiales, Harpellales, and Genistellales.

The Trichomycetes are not as rare and restricted as I feel most mycologists believe them to be. They actually are associated with a wide variety of insects, crustacea, and diplopods which can be found in most regions of the United States, and which have been collected in Central and South America, Europe, Africa, and Asia. Their habitats are terrestrial, aquatic, or marine. They have been taken from altitudes of 1800 meters on land, to depths of 40 meters in sea water. It is true that most collections around the world have been very scant and scattered, and that only some parts of Europe and the Americas have been carefully searched. But we have every reason to believe that these fungi can be found wherever potential hosts occur.

The unusual habitat of the Trichomycetes may be the circumstance that has led to their being studied by persons of various disciplines (naturalists, zoologists, parasitologists, protozoologists), but rarely by mycologists. One can find references in the literature where species of the Trichomycetes have been assigned to the algae, the sporozoans, and other areas of dubious phylogeny.

I have the impression that some mycologists still are not convinced

[1] Presented as part of the Mycological Society of America symposium, "Classification of Fungus Groups of Debatable Affinity," at the 126th meeting of the American Association for the Advancement of Science, December 30, 1959, Chicago, Ill.

of the fungal nature of the Trichomycetes, or, if they do accept this, they feel that so little is known about the group that these organisms had better be left alone until more substantial evidence has been presented to show their characteristics and affinities.

No one is more aware of the need for study on the Trichomycetes than are those few biologists actively investigating them. Nevertheless, a respectable volume of literature on these fungi has accumulated, and, turning to some publications, and a few of my personal observations, I would like to show that it is not too speculative to take these organisms and find them a tentative home in the community of fungi.

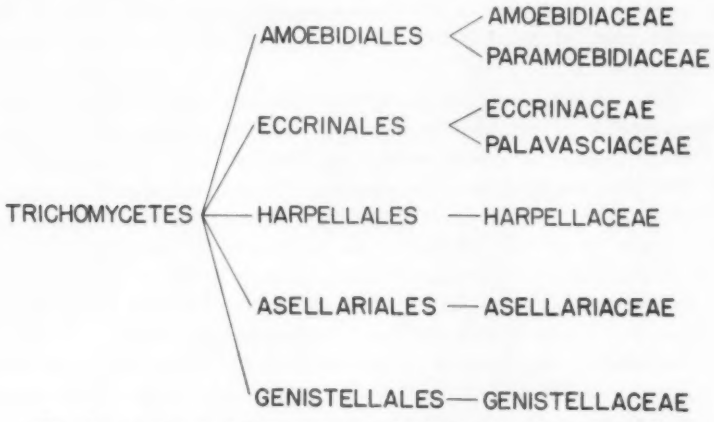

FIG. 1. A suggested scheme for the families and orders comprising the Trichomycetes.

In FIG. 1 I am suggesting a taxonomic arrangement for the Trichomycetes, consisting of five orders and seven families. This scheme differs somewhat from that suggested by the French workers. Tuzet and Manier in France during the past decade (Tuzet & Manier, **1951**, **1953**; Manier, **1955a**) have evolved several modified taxonomic arrangements for the Trichomycetes. They consider these organisms as a distinct group of fungi, despite the acknowledged similarities to some of the Phycomycetes. The latest arrangement by Manier (**1955a**) breaks down the Trichomycetes into two classes of fungi: the Prototrichomycetes containing the Amoebidiales, and the Eutrichomycetes containing the remaining fungi under two orders, the Eccrinales and the Harpellales. I suggest that the Trichomycetes need not be treated as a taxon completely distinct from the Phycomycetes.

I would like to turn first to the order Eccrinales, with perhaps two good families, the Eccrinaceae and the Palavasciaceae. The Eccrinales probably has been studied more intensively and contains more known representatives, including some of the oldest known species, than any other Trichomycetes.

ECCRINALES

Species of Eccrinales, like species of all the Trichomycetes, do not seem to have any true parasitic relationship with their hosts. Their nutrition probably comes from the surrounding materials within the gut cavity. No penetration into host tissues has ever been observed. Yet, one never finds species of Eccrinales growing elsewhere than in the digestive tract of arthropods. They may be referred to as obligate commensals.

It has not been possible to culture species of Eccrinales, or other endocommensal Trichomycetes, *in vitro* with any success for continued periods of time. There are peculiar problems involved in their cultivation that have not been solved adequately. Tuzet and Manier in France (Manier, **1954**, **1955b**; Tuzet & Manier, **1954**) have been able to get some stages of certain Trichomycetes to develop in hanging drop cultures, and I have followed very limited growth stages in some species. Most observations on species of Trichomycetes reported in the literature were made on isolated and stationary developmental stages.

The relative simplicity of a vegetative thallus belies its more fickle reproductive nature (Lichtwardt, **1958**). Since my purpose is to clarify, rather than to confuse, I will consider only its fundamental aspects of growth and reproduction.

Let us select the largest genus, *Enterobryus*, as our example of the family Eccrinaceae (FIG. 2). In this family I include what others consider to be two to four different families. Before the thallus sporulates, *Enterobryus* is a simple, coenocytic tube with a secreted, noncellular holdfast at the proximal end that causes the filament to adhere strongly to the chitinous lining of the gut (or, in a few species, also to the cuticula of the nematodes in the gut).

Asexual reproduction is by spores of two principal types. Both types are produced singly within sporangia that form successively from the apex of the filament toward the base. The spores generally escape laterally through a hole in the sporangial wall; this wall later decomposes.

One type of spore is multinucleate, with 4 or 8 nuclei at maturity, and is capable of germinating within the host which bore it, thus helping to build up the fungal population endogenously.

The other type of spore is uninucleate and sooner or later takes on an oval or ellipsoid shape and may become rather thick-walled in some species. These uninucleate spores appear to function in most cases as means of infecting other susceptible hosts that ingest them.

Sexual behavior in the Eccrinaceae has been reported in a very few species. Léger and Duboscq (Duboscq, Léger & Tuzet, **1948**) reported in *Eccrina flexilis* Lég. & Dub. (actually an *Enterobryus*) a strange and interesting "sexual" phenomenon that involved karyogamy between adjacent protoplasts within the hyphae. I will not review this process here, inasmuch as it is not very probable (even for a fungus). Manier later (**1950**) was not able to find such a phenomenon when she studied this same species.

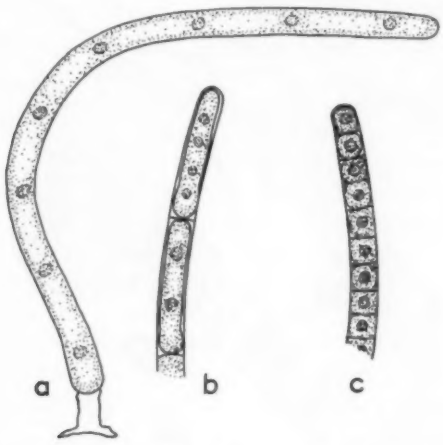

FIG. 2. *Enterobryus borariae* Lichtwardt (Eccrinaceae). a. Very young thallus. b. Hyphal tip producing multinucleate sporangiospores. c. Uninucleate sporangiospores.

Tuzet and Manier (**1948a**) reported an observation that they considered to be a sexual process in *Enterobryus duboscqui* Tuz. & Man., involving a pairing and fusion of nuclei within the coenocytic thallus, with the resulting diploid nuclei entering into the formation, within the thallus, of uninucleate, thick-walled spores that finally underwent meiosis to form 4-nucleate, haploid spores.

This sexual process is not unreasonable. However, in the many species of *Enterobryus* that I have studied, I have not yet observed any nuclear behavior comparable to this.

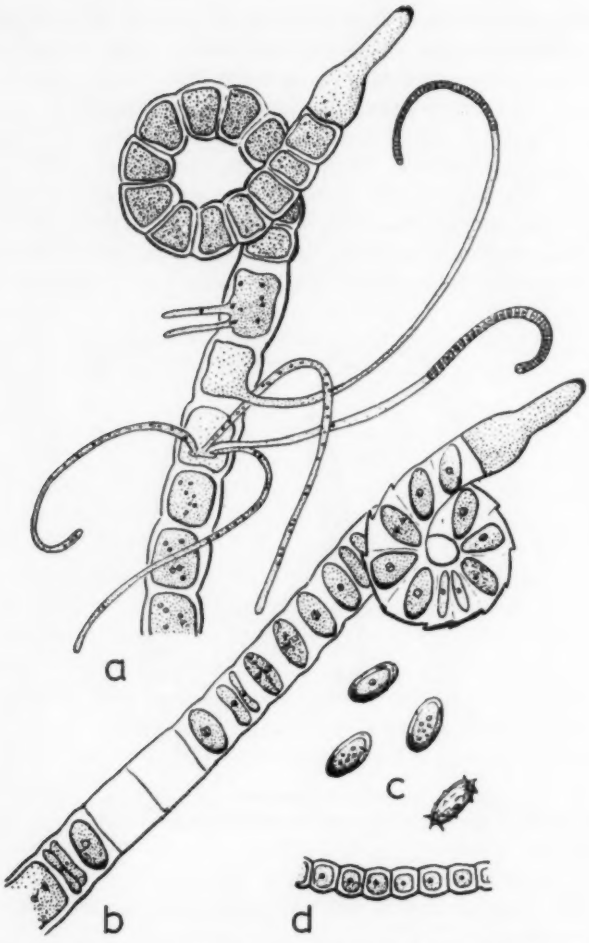

FIG. 3. *Palavascia sphaeromae* Tuzet & Manier (Palavasciaceae). a. Tip of thallus whose cells (sporangia) are germinating and producing narrow lateral hyphae. b. Tip of thallus producing sporangiospores. c. Thick-walled sporangiospores. d. Enlarged view of terminal cells produced in lateral hyphae. From Manier, 1950.

The only other family of Eccrinales that I feel can be justified at present is the Palavasciaceae. *Palavascia* (FIG. 3) is a fascinating organism in many ways, but there are only two features that I want to point out at this time.

The first feature is the way in which the sporangia may germinate *in situ* to give rise to narrow lateral filaments which in turn may produce tiny terminal cells (FIG. 3a, d).

The second feature is the production of thick-walled spores (FIG. 3c), which, according to Tuzet and Manier (**1948b**), are the result of sexual fusion of paired cells (FIG. 3b). This would be comparable to the sexual behavior that they report in *Enterobryus*. Recently I have been studying a species of *Palavascia*, from a small marine isopod, very similar in most respects to *P. sphaeromae* Tuz. & Man. (FIG. 3). I have not yet found in my species any sexuality such as Tuzet and Manier reported, despite abundant production of the thick-walled, ellipsoid spores.

It can be seen from this very brief characterization of the Eccrinales that we have not sufficient clues to help us establish good ties between the Trichomycetes and other fungi, beyond the useful information that the Eccrinales possess coenocytic thalli, and that they reproduce by means of sporangiospores. These features show closer relationship to the Phycomycetes than they do to the other classes of fungi. The sexual processes in the Eccrinales, if they have been correctly interpreted, involve no specialized gametes, gametangia, or other sexual structures that might throw light upon the systematic problems.

In other orders of the Trichomycetes, we find specific structures that are possibly indicative of relationship to the Phycomycetes. Also, we must satisfactorily demonstrate that these other orders bear some relationship to the Eccrinales. With these objectives in mind, I will consider briefly the other four orders of Trichomycetes.

AMOEBIDIALES

This order, with only the two genera *Amoebidium* and *Paramoebidium*, possesses a thallus that in its nonreproductive state may resemble very closely that of the Eccrinales (FIG. 4a), with a basal holdfast attaching the nonseptate, unbranched filament to the host. The attachment of species of *Amoebidium* to their hosts is of special interest, inasmuch as the species generally are found growing not in the digestive tract of the arthropods, but attached indiscriminately to the external parts of aquatic crustacea and insects. Because *Amoebidium* is an ectophyte and occurs on a wide range of host types, it has been the subject of study by a number of investigators. Currently *Amoebidium* is being studied in pure culture by Howard C. Whisler in Prof. Emerson's laboratory at Berkeley, Calif., and we can look forward to some interesting and exciting developments in this work.

Unfortunately, at present, one cannot find in the literature any gen-

eral agreement regarding the developmental studies of the species, except that they may produce two principal types of spores. One of these is an endospore (FIG. 4b–d) that is formed by the simultaneous conversion of the entire protoplast into a series of these spores which then emerge

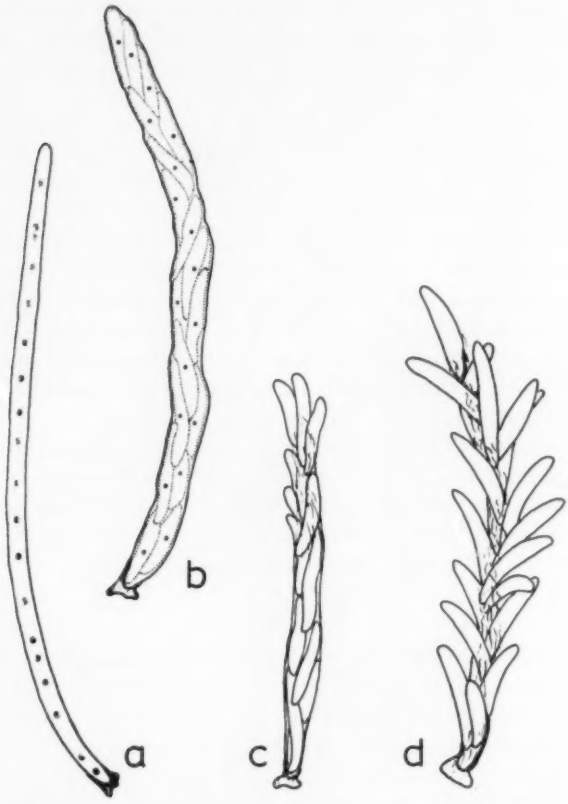

FIG. 4. *Amoebidium parasiticum* Cienkowski (Amoebidiaceae). a. Vegetative thallus. b–d. Production and release of rigid endospores. From Taylor, **1928**.

laterally or terminally. The other spore type is also an endospore, produced in a similar manner but without a definite wall (FIG. 5), and which moves about in the water by amoeboid motion. There are reports by Poisson (**1931**) and Tuzet and Manier (**1951**) that these amoebae may fuse, and, according to the latter, this fusion is a sexual process

which results in an encysted zygote which later functions as a sporangium.

Paramoebidium appears to be very closely related to the genus *Amoebidium*, differing only in that there are no rigid endospores produced—only the amoeboid type—and the various species of *Paramoebidium* occur within the guts of their hosts, and sometimes even in association with other branched Trichomycetes in the same hosts (Léger & Gauthier, **1932**; Tuzet & Manier, **1955**).

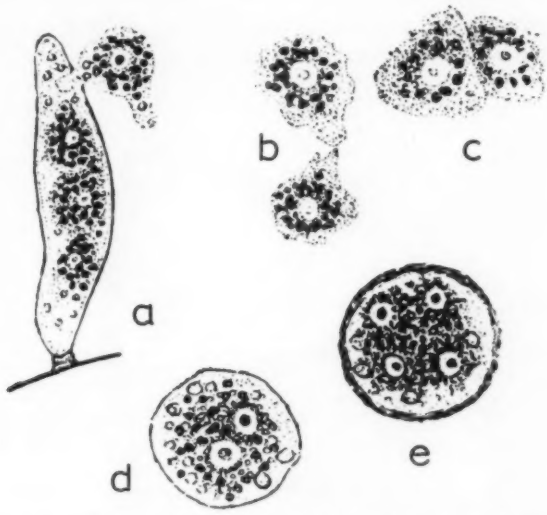

FIG. 5. *Amoebidium poissoni* Duboscq, Léger & Tuzet. a. Thallus releasing amoeboid cells. b–e. Fusion and encystment of amoeboid cells. From Poisson, **1931**.

We find in the Amoebidiales fungi that closely resemble the Eccrinales when in the vegetative state and that have the same peculiar habitat, particularly in the genus *Paramoebidium*. Reproductively, the Amoebidiales produce sporangiospores, though these apparently are not homologous with those of the Eccrinales.

It is perhaps pertinent to mention that Tuzet, Manier, and Jolivet (**1957**), in publishing on a new species of Eccrinales (*Cestodella operculata* Tuz., Man. & Jol.) describe and illustrate the production of somewhat amoeboid cells formed in large apical sporangia. These spores escape through a large terminal opening and assume a spherical shape (their fate is not known).

It may have no real bearing on the present discussion, but I cannot resist the temptation to interject a mention of a curious fungus that Cienkowski (**1861**) described as a parasite of his fungus *Amoebidium parasiticum*. He called it *Basidiolum fimbriatum* (FIG. 6), describing

FIG. 6. *Basidiolum fimbriatum* Cienk. parasitizing *Amoebidium parasiticum* Cienk. Various stages in development of parasite on host. From Cienkowski, **1861**.

it but not giving it any definite position in the fungi. Looking at his figures and the development of the fungus, does *Basidiolum* not look like a sessile *Syncephalis*, that is, without the cylindrical portion of the sporangiophore, and also without the usual prominent external rhizoids? Knowing the predilection of the Piptocephalidaceae to parasitize mem-

bers of the Mucorales, I leave you with any assumptions you may care to make.

HARPELLALES

The young thallus of a *Harpella* bears a very close resemblance to that of genera in the Eccrinales and Amoebidiales (FIG. 7a). It is filamentous, nonseptate, and unbranched, bearing a holdfast at the basal end. When the thallus matures, it remains unbranched, but becomes septate during the beginning stages of sporulation. Instead of producing sporangiospores internally, as the Eccrinales and Amoebidiales do, *Harpella* produces a short lateral branch from each cell, a lateral conidium (FIG. 7b, c). This conidium presumably is the only means of asexual reproduction in the Harpellales.

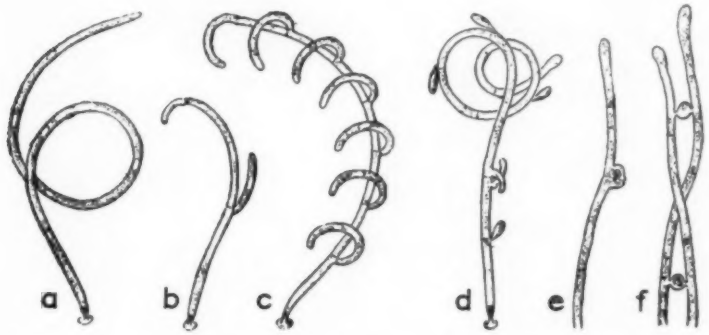

FIG. 7. *Harpella melusinae* Léger & Duboscq (Harpellaceae). a. Vegetative thallus. b, c. Production of lateral conidia. d, e. Conjugation of adjacent cells of same thallus by means of short lateral projections. f. Conjugation of two thalli. From Léger & Duboscq, **1929**.

Léger and Duboscq, in establishing this new genus in 1929, also described what appeared to be sexual fusion between adjacent cells in the same hypha (FIG. 7d, e), through short, lateral conjugating processes, and also by the conjugation of adjacent hyphae (FIG. 7f). These authors did not actually describe the mature zygospore, but they did state that in many preparations they observed thick-walled, uninucleate spherical bodies lying loose near the hyphae that might have been the zygospores of their *Harpella*. There is a similarity between this type of reproductive process (as Léger & Duboscq, **1929**, and Léger & Gauthier, **1932**, have noted) and that described by Eidam for *Basidiobolus ranarum* Eidam in the Entomophthorales (**1887**).

Tuzet and Manier (**1955**) in their studies of *H. melusinae* Lég. & Dub. var. *eyziesi* Tuz. & Man. reported a slightly modified type of conjugation, wherein the neighboring cells of a thallus fused without the formation of the short lateral beaks (FIG. 8d, e).

In *Harpella* we find what appears to be the production of zygospores by the conjugation of adjacent cells in the same thallus, or of cells in different thalli. The formation of zygospores is one of the fundamental characteristics of the Zygomycetes.

The similarity of a young *Harpella* and a thallus of one of the Eccrinales already has been pointed out. Reproductively, *Harpella* differs

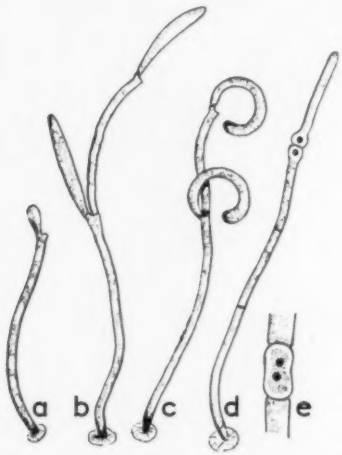

FIG. 8. *Harpella melusinae* var. *eyziesi* Tuzet & Manier. a–c. Thalli with lateral conidia. d, e. Conjugation of adjacent cells of same thallus without lateral projections. From Tuzet & Manier, **1955**.

significantly from species of Eccrinales by producing lateral asexual spores and zygospores.

ASELLARIALES

Upon first observation, the Asellariales, with the single genus *Asellaria,* would appear to have no particular close relationship to the Eccrinales, for the growth habit of species of *Asellaria* shows them to be highly branched, septate, and directly attached to the host's gut lining by means of an oddly-shaped basal cell (FIG. 9).

No sexual reproduction is known in the order.

In reproducing asexually, various branches may segment to form a series of uninucleate cells which may then break away and presumably germinate later to form new thalli (Poisson, **1932**). In other species, a definite single body forms within these cells.

Recently I have been studying a new species of *Asellaria* that occurs within the hindgut of an isopod that lives in the intertidal zone along the coast of North Carolina. This species produces cells which either

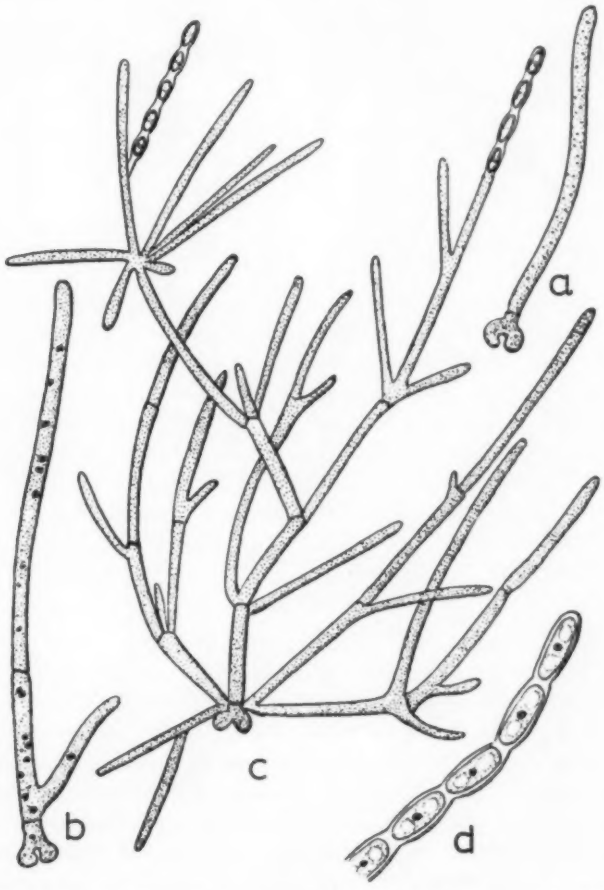

FIG. 9. *Asellaria ligiae* Tuzet & Manier (Asellariaceae). a, b. Developing thalli showing basal cell which functions as a holdfast. c. Mature thallus. d. Spores. From Manier, **1950**.

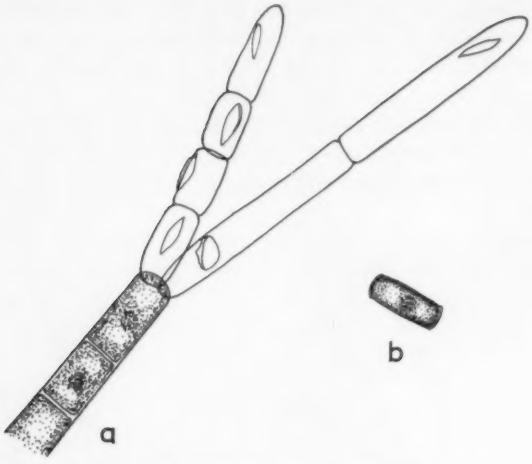

FIG. 10. *Asellaria* sp. from *Ligia exotica*. a. Empty sporangia. b. Sporangiospore.

break away like arthrospores, or it forms the type of enclosed body referred to above. I was very much interested to find that these structures are sporangiospores that emerge from a lateral opening in the sporangial wall (FIG. 10) very much in the manner that occurs in species of Eccrinales. One might interpret the arthrospores which these fungi produce as being sporangia that disarticulate without producing spores, but which are capable of germinating and forming new thalli. I occasionally have found this same phenomenon of sporangial dehiscence in a few species of *Enterobryus*, though I considered such cells to be nonfunctional oddities.

GENISTELLALES

This fifth order of the Trichomycetes possesses some features of both the branched Asellariales and the unbranched Harpellales.

Like the Asellariales, the thalli of the Genistellales are branched, septate, and possess a cellular holdfast (FIG. 11). In the genus *Spartiella*, unlike *Stipella*, the cell that anchors the thallus to the gut lining is reminiscent of the holdfast in *Asellaria*, and different modifications of these basal cells are found in other genera of Genistellales.

Reproductively, the Genistellales show far greater similarities to the unbranched Harpellales than they do to the branched Asellariales.

The Genistellales produce what apparently are zygospores formed by the conjugation of adjacent branches of a common thallus (FIG. 11). The method of their formation would appear to be very similar to what

has been reported in various species of *Entomophthora* (*Empusa*), though the shape of the zygospores is quite different from the spherical zygospores of the Entomophthorales.

The Genistellales also produce lateral conidia that are like those of species of *Harpella,* though in several genera (i.e., *Spartiella,* FIG. 12)

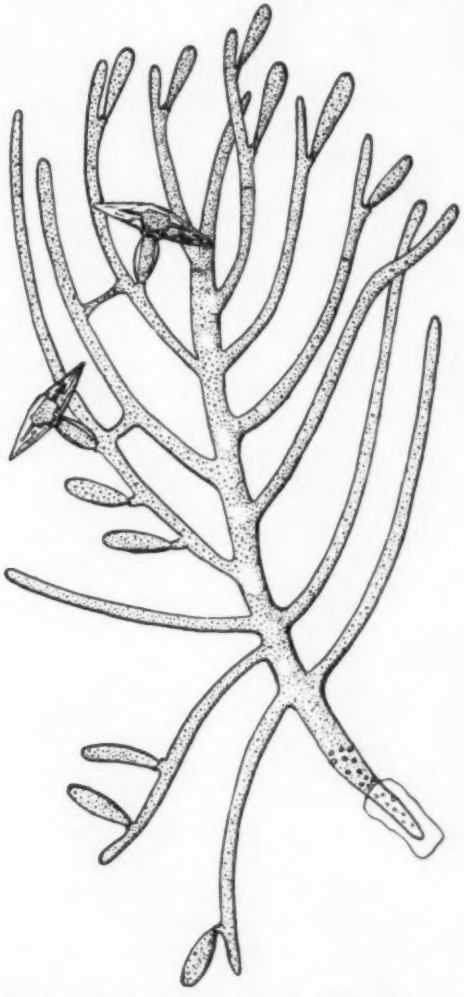

FIG. 11. *Stipella vigilans* Léger & Gauthier (Genistellaceae). Thallus bearing lateral conidia and zygospores. From Manier, 1950.

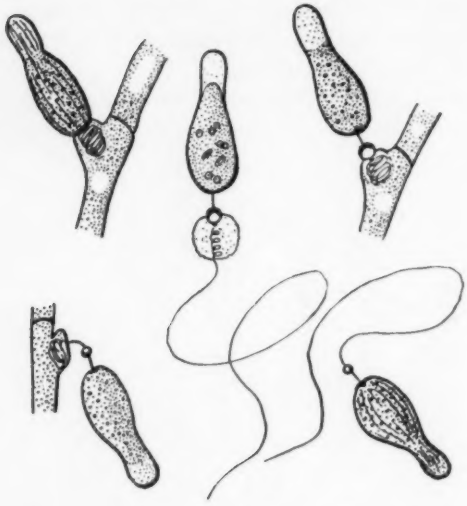

FIG. 12. *Spartiella barbata* Tuzet & Manier (Genistellaceae). Lateral conidia with appendage. From Manier, **1950**.

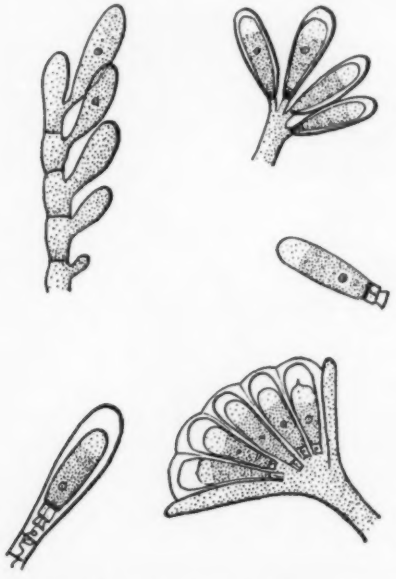

FIG. 13. *Orphella culici* Tuzet & Manier (Genistellaceae). Lateral and terminal "conidia" (sporangiospores?). From Manier, **1950**.

a strange fine threadlike appendage, sometimes coiled before release of the spore, has been seen attached to the proximal end of the conidia. There is no indication that these function as organs of motility. They would be analogous to, but not homologous to, the spores of some of the Choanephoraceae. Léger and Gauthier (**1935**) reported the finding in *Harpella* of somewhat similar spores with one to several appendages at one end of the spores. Other authors (Manier, **1950**; Tuzet & Manier, **1955**) have not reported appendaged spores in *Harpella.*

FIG. 13 shows the conidia of *Orphella culici* Tuz. & Man. described in 1947. According to the description and figures of Tuzet and Manier (**1947**), one can infer that the structures are actually lateral (or terminal) sporangia containing a single spore within each sporangium.

Is it possible that these so-called "conidia" (now referred to by Manier (**1955a**) as "mastigocystis") of *O. culici* are really sporangiospores? Is it not possible to consider that the other "conidia" of the Genistellales, as well as those of the Harpellales, might be phylogenetically reduced sporangia whose protoplasts do not develop a distinct inner spore? There exists good evidence that the "conidia" of some of the Zygomycetes (or Peronosporales) may have arisen by reduction of sporangia. Such a suggestion in the Trichomycetes cannot be answered on the basis of our present knowledge, but it would be well if researchers would keep this question in mind. Should it turn out that these lateral "conidia" are in effect sporangia, then the sporangium is a feature of all the orders of Trichomycetes.

FIG. 14 illustrates some of the characteristics of the various orders of Trichomycetes.

Professor Ralph Emerson reported at the 1958 AIBS meetings at Stanford, and, with Mr. Howard C. Whisler, at the 1959 International Botanical Congress, upon the eccrinid-like fungus *Oedogoniomyces,* originally described by Kobyashi and Ookubo (**1954**) in Japan. Dr. Emerson's isolates were made from submerged apples and other fruits in Costa Rica, and he has easily obtained the fungus in pure culture. This remarkable fungus has some of the appearance of an Eccrinaceae, being a simple filament with a basal holdfast, but it produces apically a succession of sporangia within which are formed posteriorly uniflagellate zoospores. If such a fungus were to be related to the Eccrinales, or to some other Trichomycete order, it would be very interesting. *Oedogoniomyces* as a Trichomycete would simply show further affinities to, or parallelisms with, other Phycomycetes, in this case to the aquatic series, rather than to the Zygomycetes.

Our present knowledge of the Trichomycetes indicates that their evolutionary development may not have been recent. As evidence I

cite: 1. Their geographical distribution which in all probability is very wide. 2. The great range in types of arthropod hosts and host-habitats. 3. The variation of vegetative and reproductive structures that have evolved in the different orders of the Trichomycetes.

I do not care to speculate on phylogenetic relationships, either within the Trichomycetes, or between the Trichomycetes and other Phycomycetes. (I feel that Sörgel's paper (**1953**), on the phylogeny within the

TRICOMYCETES	AMOEBIDIALES	ECCRINALES	HARPELLALES	ASELLARIALES	GENISTELLALES
THALLUS SIMPLE	X	X	X		
THALLUS BRANCHED				X	X
HOLDFAST NON-CELLULAR	X	X	X		
HOLDFAST CELLULAR				X	X
SPORANGIOSPORES	X	X		X	
LATERAL "CONIDIA"			X		X
ZYGOSPORES			X		X

FIG. 14. Summarization of characteristics found in the orders of Trichomycetes.

Trichomycetes is premature, and I do not believe his lines of evidence are good.) In view of the possible evolutionary age of the Trichomycetes, it is just as feasible to think of them as having evolved in some way parallel to the higher Phycomycetes as it is to suppose that they have evolved from the Zygomycetes.

Over two dozen persons during the past 110 years have made contributions to our knowledge of the Trichomycetes. There still is confusion in much of the terminology and taxonomy of the group, and too

many observations have been superficial and sometimes erroneous. These difficulties reflect the varied biological backgrounds of the investigators and the hardships inherent in studying these organisms. But the groundwork has been laid, especially by a number of French workers (Tuzet, Manier, Léger, Duboscq, Poisson).

The taxonomic position of the Eccrinales and related fungi will be more definitive only when a larger number of biologists devote more time to finding, studying, and describing what they find. This information is fundamental to many of the fascinating biological, ecological, and physiological problems still to be solved in this unique group of microorganisms.

CONCLUSIONS

1. The Eccrinales and other arthropodophilic fungi known as the Trichomycetes justifiably can be included among the true fungi (Eumycophyta).

2. Despite the diversity of form and reproductive structures found in these fungi, there may be sufficient relationships among the different orders to warrant considering the Trichomycetes as an integral taxon.

3. The general vegetative and reproductive characteristics of the Trichomycetes (coenocytism, sporangia, zygospores) indicate that they have decided affinities to the Phycomycetes.

4. The Trichomycetes have some reproductive features in common with the Zygomycetes (Aplanatae), but our present concepts of this subclass do not permit our incorporation of the Trichomycetes in the Zygomycetes.

5. The Trichomycetes at present are best treated taxonomically as a subclass of the Phycomycetes.

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RESTING SPORES IN PHLYCTOCHYTRIUM PLANICORNE

CLYDE J. UMPHLETT AND MARGARET M. HOLLAND

(WITH 15 FIGURES)

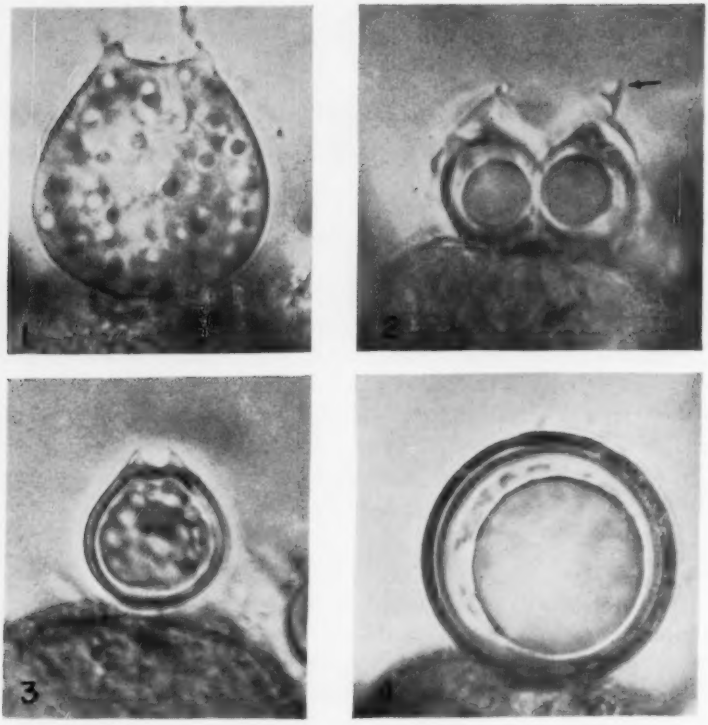
In February, 1960, a soil and water sample taken from an area in Chapel Hill, N. C., known locally as Meeting of the Waters, was baited for chytrids with the pollen of *Pinus taeda* L. and *Liquidambar styraciflua* L. After a week zoosporangia of the dentigerate chytrid, *Phlyctochytrium planicorne* Atkinson, were growing on the floating pollen grains of *Pinus* and *Liquidambar*. Examination revealed abundant young resting spores. This paper is the first report of resting spores in this species.

DEVELOPMENT OF THE RESTING SPORE

Development was not observed as a continuum on one thallus, but sequences were studied by periodically mounting in water on a microscope slide thalli growing on pollen grains.

In the early stage (FIG. 7) the thallus is composed of an epibiotic portion in which the epibiotic resting spore will be produced, and an endobiotic spherical to subspherical apophysis. Rhizoids were observed in later stages, but their early presence is uncertain because of the dense interior of the pollen grain. The epibiotic portion in the very young stage is ovoid to pyriform and has at its apex four blunt protuberances (only two show in FIG. 7). Cytoplasm extends from the lower expanding body of the developing epibiotic portion into the apical protuberances. A single, large, conspicuous, refractive globule and a few smaller ones are in the otherwise homogeneous cytoplasm of the extramatrical body. The wall is thin. Apparently the thallus arose from a zoospore.

In a later stage of the resting spore (FIG. 8), both the epibiotic and endobiotic portions of the thallus have enlarged considerably. The nearly spherical apophysis bears one or more slender, tapering rhizoids which, however, could not be traced to their terminations because of the density of the substratum. The epibiotic body possesses now four solid teeth devoid of cytoplasm probably because of the deposition of wall material. The wall is still thin at this stage, and the greatly enlarged



FIGS. 1–4. *Phlyctochytrium planicorne*. FIG. 1. Mature zoosporangium. FIG. 2. Two young resting spore thalli developing inside the wall of a previously discharged zoosporangium; one tooth on the old sporangium indicated by arrow. FIG. 3. Immature resting spore thallus with a single-layered thick wall. FIG. 4. Mature resting spore with its own wall inside the container wall of the epibiotic portion of the producing thallus. All × 1800.

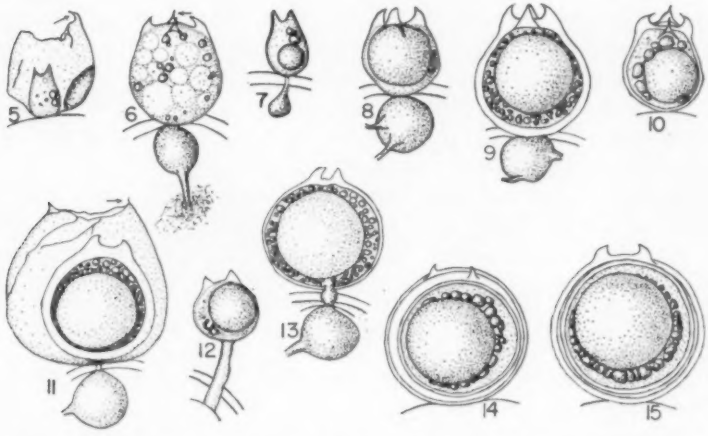
refractive globule is surrounded by cytoplasm. The cytoplasm is mostly homogeneous, but has denser areas and a few small refractive globules (FIGS. 8, 12).

Little or no change occurs subsequently in the endobiotic system. Apparently development of the apophysis and rhizoids is completed by the time the wall of the epibiotic body thickens. In late developmental stages the apophysis is optically empty, indicating that a transfer of the material from the endobiotic system to the epibiotic body occurs. It is probable that the endobiotic and epibiotic portions of the thallus are separated at maturity by a septum, but it was not seen.

The epibiotic portion of the thallus increases in size. Its wall thickens to as much as 4 μ, and is single-layered. The cytoplasm between the large refractive globule and the wall contains many small refractive globules 1–2 μ in diameter. At this stage the small globules are dispersed randomly (FIGS. 9, 11, 13). Stages representing a transition from that just described to the mature resting spore were not observed.

There are striking similarities in the external morphology of the developing resting spore thallus (FIGS. 3, 9–11, 13) and the zoosporangium (FIGS. 1, 5, 6). Both possess the apical teeth and basic ovoid shape so characteristic of the species, and both show a well defined apophysis bearing rhizoids.

The epibiotic portion of the resting spore thallus at maturity is a spherical to subspherical structure exhibiting an inner and an outer wall with a clear area between the two walls. The outer wall, 1.5–2.0 μ thick, becomes light yellowish tan, and has at its apex four solid teeth



FIGS. 5–15. *Phlyctochytrium planicorne*. FIG. 5. Very young zoosporangium developing inside empty zoosporangium wall; note needlelike attenuation on tooth indicated by arrow. FIG. 6. Immature zoosporangial thallus with highly vacuolate cytoplasm; note attenuations on teeth. FIG. 7. Very young resting spore thallus showing large refractive globule and apophysis. FIG. 8. Developing resting spore thallus with thin wall. FIG. 9. Immature resting spore thallus with thickened wall and prominent teeth. FIG. 10. Resting spore thallus with needlelike attenuations on teeth. FIG. 11. Immature resting spore thallus developing inside wall of previously discharged zoosporangium; note attenuation on tooth of empty sporangium. FIGS. 12, 13. Stalked forms of resting spore thalli. FIGS. 14, 15. Mature resting spores inside containers. Drawings made with the aid of camera lucida. All $\times$ 1000.

typical of the zoosporangium, but less prominent on the mature resting spore (FIG. 14). The inner wall is smooth, 1–2 μ thick, colorless, and includes the large, eccentric, refractive globule, 9–15 μ in diameter, surrounded by a layer of smaller refractive globules appressed to its periphery. The small area between the layer of small globules and the inner wall is occupied by homogeneous cytoplasm. The structure composed of the inner wall and its contents is interpreted as the resting spore itself. Germination of the resting spore has not been seen.

Several zoosporangia and resting spore thalli developed inside the walls of previously discharged zoosporangia (FIGS. 5, 11). Possibly these developing structures were the products of internal proliferation. However, empty zoosporangial walls were seen with more than one thallus growing inside (FIG. 2), indicating that the internal thalli developed from zoospores which had come to rest in those places.

Other resting spore thalli were attached to the substratum by slender stalks, 2–3 μ wide, and up to 10 μ long (FIGS. 12, 13). Apparently the parental zoospores had germinated some distance from the pollen grains, but had penetrated them. Development of the epibiotic portion away from the substratum produced the stalked structure.

The teeth on some resting spore thalli were terminated by needlelike attenuations (FIG. 10). Attenuations were common on zoosporangia (FIGS. 5, 6, 11), but less frequent on resting spore thalli.

In view of this new information the diagnosis of *Phlyctochytrium planicorne* can be emended. The diagnosis of the zoosporangial phase has been drawn largely from the literature (Atkinson, **1909**; Sparrow, **1932**, **1938**), but our observations are included.

Phlyctochytrium planicorne Atkinson emend.

Thallus monocentric, eucarpic. Zoosporangium epibiotic, ovoid to broadly ovoid, 8–17 μ wide × 6–24 μ high, inoperculate, with an apical collarette of 4 (rarely 6) plain, solid teeth surrounding and slightly converging over the apical discharge pore; teeth up to 4 μ high, sometimes terminated by needlelike attenuations; zoospores discharged through an apical pore in a mass surrounded by a vesicle or discharged singly, spherical, 3–6 μ in diameter, with a few minute droplets and a spherical, slightly eccentric refractive globule; flagellum single, posteriorly directed, ca. 30 μ long; endobiotic system composed of a spherical or subspherical apophysis, 4–13 μ in diameter when spherical, up to 12 μ wide, 10 μ high when subspherical, bearing slender, tapering branching rhizoids. Resting spore thallus with epibiotic and endobiotic portions; epibiotic part at maturity composed of a spherical to subspherical container, 15–26 μ in diameter, container wall 1.5–2.0 μ thick, becoming

light yellowish tan with age, ornamented with 4 teeth like the zoosporangium, enclosing the resting spore; resting spore spherical or nearly so, 11–20 μ in diameter, wall 1–2 μ thick, separate from container wall, smooth, colorless, without ornaments, containing a single, large, eccentric refractive globule 9–15 μ in diameter, and a layer of small refractive globules appressed peripherally to the large one; endobiotic system as in zoosporangial thallus; development apparently asexual from a zoospore; germination not observed.

DISCUSSION

Phlyctochytrium planicorne is the only member of the genus that has its resting spore, at maturity, lying in a container ornamented like the zoosporangium. Resting spores have been reported for two ornamented species of *Phlyctochytrium*, *P. chaetiferum* Karl. (Karling, **1937**), and *P. desmidiacearum* Dang. (Dangeard, **1937**). The zoosporangium of *P. chaetiferum* is bedecked with long, flexible, branching hairs, but the resting spore has a smooth, single-layered wall without ornaments, develops asexually, and is epibiotic. *P. desmidiacearum* zoosporangia are decorated apically with a collarette of four bluntly bipartite teeth. The resting spores are spherical, extramatrical, have a thick, single-layered wall without teeth, and are formed asexually. The report of resting spores in this species is the first for a species of the *Dentigera* section of *Phlyctochytrium*. Resting spores have been observed by W. J. Koch (personal communication) in a second dentigerate species, *P. bullatum* Sparrow.

In his discussion of chytrid resting spores, Sparrow (**1943**) points out that, in resting spores having two walls or one wall of two layers, the outer wall "probably represents the wall of the container." The container of the resting spore of *P. planicorne* is the original wall of the epibiotic portion of the resting spore thallus. It is not clear, however, how the wall of the resting spore becomes distinct from the original (container) wall. Sparrow (**1943**), in discussing the resting spore of *Olpidium gregarium* (Nowak.) Schroeter, suggests that the resting spores might arise by a contraction of the protoplasm followed by the formation of a wall around the protoplasm. Scherffel (**1926**) suggests a contraction process also in *Olpidium saccatum* Sorokin and *Chytridium olla* Braun. Such a process is unlikely in *P. planicorne*, however, since a comparison of mature resting spores with the largest immature resting spore thalli shows that the spore inside the container (FIGS. 14, 15) occupies as much volume as the whole epibiotic portion of the immature thallus (FIG. 13). It is more likely that the container wall expands,

possibly pulling away from the enclosed protoplasm, and the protoplast then lays down a new wall to form the separate wall of the resting spore. The increase in size of the resting spore supports such an occurrence as does the expansion of the container wall at the expense of its thickness (FIGS. 9, 11, 14, 15). The wall of the container enclosing a mature resting spore is thinner than that of a thallus in the later stages prior to maturation. The nature of the teeth on the mature wall indicates that a stretching process occurs in the container wall. The teeth are not so prominent as on younger resting spore thalli. It appears that, in the expansion of the wall, portions of the bases of the teeth are stretched and flattened along the surface of the wall and are recognizable as a part of the wall rather than as portions of the ornaments.

SUMMARY

Resting spores are reported and described in the dentigerate chytrid, *Phlyctochytrium planicorne* Atkinson. The resting spore is epibiotic, and during development is similar in external morphology to the zoosporangium of the species. Both are ovoid and have four plain, solid teeth forming a collarette around the apex of the thallus. During development of the resting spore thallus, which is apparently asexual from a zoospore, a single-layered thick wall is laid down by the extramatrical portion. Enclosed by this wall is a single large, conspicuous, refractive globule surrounded by several smaller ones. At maturity the resting spore is spherical or nearly so, has its own distinct, smooth wall, and lies within a light yellowish tan ornamented container probably formed from the original wall of the extramatrical portion of the producing thallus. The large globule of the mature spore is eccentric with a layer of smaller globules tightly appressed to its periphery. The endobiotic portion of the resting spore thallus is similar to that of the zoosporangial phase. Germination of the resting spore was not observed. An emended diagnosis of *Phlyctochytrium planicorne* is presented.

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SYNCHYTRIUM PILIFICUM IN AMERICA [1]

JOHN S. KARLING

(WITH 14 FIGURES)

Synchytrium pilificum Thomas is a short-cycled species which parasitizes *Potentilla tormentilla* in Europe and produces resting spores in composite hairy galls. Accordingly, it appears to be a member of the subgenus *Pycnochytrium*. The galls which it induces on its host are probably the most characteristic of any species of *Synchytrium*, and the species is readily identifiable by this character. They consist of a subhemispherical base from whose apex tufts of hairs arise and give them a hairy or felty appearance. For this reason the galls are referred to as felt galls (Küster, **1911**).

Thomas (**1883**) recognized it first as a fungus in 1869 on Alexander Braun's herbarium specimens of *P. tormentilla* which G. Engleman had collected at Frankfurt, Germany, in 1825 and 1826. In 1880 Thomas found it only once on all of the above-ground parts of the same host in Thuringia, and in 1883 he described it as a new species of *Synchytrium*. He believed that the so-called "Phytocecidium" which Low (**1883**) described on *P. erecta* relates to the galls induced by *S. pilificum*. Low based his description on specimens which had been collected in southern Bohemia in 1863 by L. Kirschner who described the galls very briefly. Schroeter (**1885**) collected it in Silesia on *P. silvestris*, and later Zopf found it on *P. tormentilla* near Münster and distributed herbarium specimens of it (Zopf, Krypt. exs. 1340). Tobler (**1912**) also reported it on *P. tormentilla* from Westphalia. She found only resting spores in June, but in February of the following year the spores had germinated to form sori and sporangia. Tobler, however, did not determine whether the spores are transformed directly into sori of sporangia, or function as prosori in germination and develop externally attached sori of sporangia. She reported only that "die Sori waren nun zum Teil in Sporangien zerfallen, und zwar 15–20." Küster (**1911**) apparently collected this species also because he illustrated a longitudinal section of a gall. Also, specimens in the Berlin-Dahlem herbarium indicate that L. Ludwig (5–6–1915) collected it on the same host in Poland. These

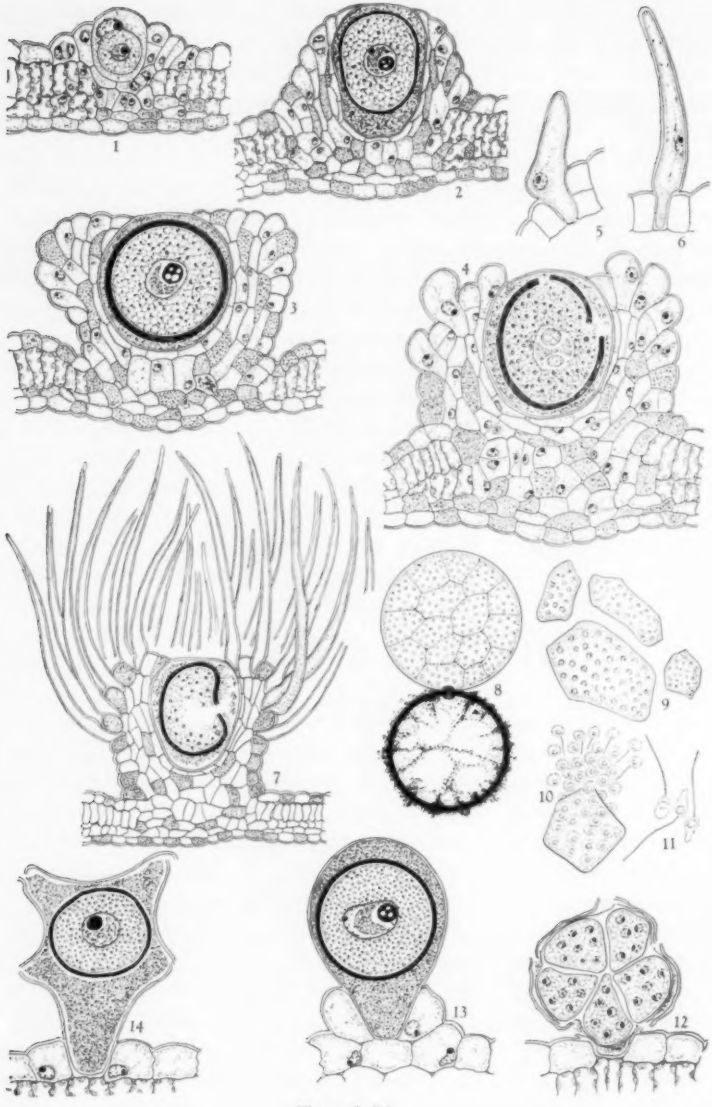
[1] This study has been supported by a grant from the National Science Foundation.

reports show that *S. pilificum* occurs widely in Europe, but up to the present time it has not been found in the western hemisphere.

I found it on *Potentilla simplex* in a refuse dumping area about 100 yards north of the Pennington School for Boys, Pennington, N. J., on June 12, 1959. This locality slopes slightly to the north and is flooded occasionally by the spring and summer rains. A large number of plants in this area were densely infected in June, 1959 and had such a striking appearance that they could be recognized at once. The infected leaves, petioles and runners were curled, wrinkled, distorted, thickened and fleshy, and the plants were markedly stunted in growth. I visited the same locality on Sept. 2, 1959 for the purpose of collecting mature resting spores for germination studies and found that the plants were as densely infected as in June. In addition to mature spores and galls on dead leaves, abundant young and developing stages of the parasite occurred on young and growing host plants. This suggested that the resting spores from the previous year germinate during the summer when sufficient moisture is present. A third visit was made to this locality on Feb. 19, 1960. Most of the host plants under the snow were dead, but a few were still green, alive and infected with developing stages of *S. pilificum*. The presence of numerous young galls without hairs indicated that spores had germinated and infected the hosts. Again in July of 1960 I found it abundantly on the same host at Gettysburg, Pa., and near Solomons, Md., which indicates that it may be widely distributed in America.

On all of the visits to Pennington infected plants were dug up and transferred to the greenhouses at Purdue University where the progress of infection and development could be studied more readily and intensively. As new leaves developed and expanded, they were found to be infected also, and from such leaves the early developmental stages of the parasite and the response of the host to infection were studied. The present contribution concerns the problems of the life cycle and development of the parasite, host reaction, and the development of the unusual hairy and composite galls.

As in other species of *Synchytrium* the planospores of *S. pilificum* come to rest on the epidermal cells of the host following a period of motility. Infection occurs in from 6 to 8 hours, and the young parasite is usually found in the base of the host cell on the second day following infection. All above-ground organs are susceptible, including both surfaces of the leaves, petioles and stolons. The early stages of infection and development of the parasite are similar to those reported for other species and need not be described in detail again. The host



FIGS. 1–14.

FIGS. 1–11. *S. pilificum.* FIGS. 11–14. *Synchytrium* sp. FIG. 1. Young parasite in enlarging host cell. × 74. FIG. 2. Later stage of gall development. × 74. FIG. 3. Gall prior to development of epidermal hairs. × 74. FIG. 4. Gall

responds to the presence of the parasite by a marked enlargement of the infected cell. At the same time the epidermal, palisade and mesophyll cells enlarge and divide. FIG. 1 shows the enlarged infected cell with the globular parasite lying beneath the host nucleus. The adjacent epidermal cells have enlarged and elongated outward, and the presence of several thin walls in this section indicates that some of the cells have divided recently. Also, one epidermal cell and another cell derived from the palisade layer are in the telophases of mitosis. These enlarging and dividing uninfected cells are the rudiments of the sheath which will envelop most of the infected cell as the gall develops. By further division and multiplication of these cells and continued enlargement of the infected cell and parasite the incipient gall becomes mound-shaped as is shown in FIG. 2. Here, also, one of the epidermal cells is in the telophase of mitosis.

In fixed and stained sections many of the sheath cells are filled with a densely stained resin-like substance which obscures the nucleus and remainder of the cytoplasm. The presence of this substance, apparently, is not a response to infection because it is present in numerous cells of uninfected leaves also. However, it appears to be more abundant in the sheath of the galls than elsewhere in the leaf.

The gall continues to develop in the manner related above until it reaches the size shown in FIG. 3. At this stage the galls may vary from 130–170 μ high by 175–208 μ broad with a sheath 3 to 6 cells thick. They may be broadly columnar to slightly obpyriform in shape with a flattened apex. Approximately $\frac{1}{5}$ to $\frac{1}{4}$ of the infected cell may be unenveloped or exposed at the apex (FIGS. 2–4). So far there is no evidence of the hairs which give the galls their characteristic shape and appearance, and at this stage the galls resemble the composite ones induced by many other species of *Synchytrium*.

The first evidence of the development of the hairs is the outward elongation and enlargement of numerous epidermal cells around the upper part of the gall as shown in FIG. 4. Such cells usually have a conspicuous nucleus and fairly dense cytoplasm (FIG. 5). As they continue to elongate their basal end usually becomes compressed by the

showing early stage of development of hairs from apical epidermal cells. × 74. FIGS. 5, 6. Stages in development of epidermal hairs. × 74. FIG. 7. Mature gall in longitudinal section. × 47. FIG. 8. Germinated resting spore. × 87. FIG. 9. Variations in sizes of sporangia. × 160. FIG. 10. Dehiscing sporangium. × 180. FIG. 11. Planospores. × 300. FIG. 12. Simple sporangial gall with sporangia. × 160. FIG. 13. Simple resting-spore gall with spore and enveloping residue. × 160. FIG. 14. Collapsed resting-spore gall. × 160.

adjacent epidermal cells (FIG. 6). Eventually, the hairs attain a length of 80–375 μ and 12 to 24 μ in diameter with a tapering apex, and curve slightly inward and over the infected cell. Accordingly, the hairs may be markedly longer than the height of the remainder of the gall (FIG. 7). The number of hairs per gall in the New Jersey specimens varies from 50 to 165, whereas only 20 to 35 hairs per gall are present in the European material according to Thomas and Tobler. These workers reported that the hairs may be up to 900 μ long by 70 μ in diameter. There are, thus, marked differences in number, length and diameter of the hairs in the American and European material, but whether such differences in host reaction are of diagnostic significance as far as the parasite is concerned is open to question. I am inclined to believe that they may vary for each susceptible host.

Nevertheless, the dense development of these hairs is a distinct reaction of this host to infection because trichomes occur only sparingly on the normal leaves. Whether or not a similar reaction will occur on other hosts has not been determined, and it remains to be shown by cross inoculation experiments that this type of a gall is characteristic of *S. pilificum* on all hosts it might infect.

In longitudinal sections, the mature galls have the structure shown in FIG. 7. Such galls are from 180–212 μ high by 213–238 μ broad, exclusively of the hairs and have a sheath 4 to 7 cells thick. However, it should be noted that a few galls lack hairs, or the hairs are short and poorly developed. Such galls may have the appearance of the one shown in FIG. 4. Spores from such galls germinate as readily as those in hairy galls which indicate that hairless galls are not immature as far as the spores are concerned. Occasionally, the base is slightly constricted so that the galls are somewhat obpyriform with a flattened apex. When they occur on a vein or the midrib, vascular strands may extend up into the base of the galls.

The resting spores are subspherical, 75–240 μ, to ovoid, 80–130 × 126–210 μ, in shape with light to golden-yellow content and an amber-brown wall, 3.8–4.6 μ thick. Usually, they do not fill the host cell completely and are enveloped by a layer of reddish-brown residue (FIGS. 3–5, 7). Spores which were collected on Feb. 19, 1960 and placed in charcoal-treated water on Feb. 22 on the laboratory table germinated on Feb. 27, and in this process they functioned as prosori (FIG. 8). The content migrated or grew out to form a vesicle in the apex of the gall. This vesicle is transformed into a sorus of sporangia and sits as a brilliantly yellowish-orange globule among the apical hairs. The sori are subspherical, 70–255 μ, ovoid, 90–230 × 140–264 μ thick and bear

60 to 520 sporangia. These are dark reddish-orange, polyhedral, and vary usually from 17 to 34 μ in diameter (FIG. 9). Under these laboratory conditions cleavage in the sori is quite unequal so that sporangia up to 70 μ in greatest diameter occur frequently. The sporangia (FIG. 10) produce approximately 150 to over 1000 planospores which are rather small, 2–2.3 × 4–4.2 μ, elongate with a tapering anterior end when actively motile and a flagellum, 10–12 μ long (FIG. 11). Under the laboratory conditions of this study sporogenesis occurred only sporadically. Usually the process proceeded until planospore initials were delimited, but only a few of the sporangia dehisced and liberated active planospores. Accordingly, relatively few planospores were obtained for cross inoculation studies. The present study, nonetheless, solves the question of how the resting spores germinate and proves that *S. pilificum* is a member of the subgenus *Pycnochytrium*.

In connection with *S. pilificum* I am reporting the occurrence of a species which parasitizes *Potentilla canadensis*. It was collected at Richmond, Va., in August, 1954, and after a preliminary examination of the living material I identified it as *S. potentillae* (Schroet.) Lagerh. because of its host and the presence of resting spores in simple galls. Since that time, however, a study of fixed and stained sections of this material has shown that this species develops sporangial galls, sori, and sporangia (FIGS. 12–14) in addition to resting spores. Obviously, it is a long-cycled species and quite different from *S. potentillae* in this respect. From the information at hand it may be diagnosed as follows:

Sori solitary, subspherical 35–48 μ, to ovoid, 38–46 × 44–46 μ, with thin hyaline walls. Sporangia 8 to 24 per sorus, polyhedral, 16–30 μ in greatest diameter. Zoospores unknown. Resting spores usually solitary, rarely 2 in a cell, spherical, 70–96 μ, or ovoid, 60–66 × 70–84 μ, with a smooth wall 3.2–4 μ thick; enveloping residue lavender-red, usually abundant and filling remainder of gall; germination unknown.

Simply dihomeogallic, galls usually scattered on both surfaces of leaves and causing little or no distortion. Sporangial galls small, subspherical, 45–62 μ to ovoid. Resting-spore galls usually obpyriform, 84 to 156 μ high and 68 to 132 μ at broadest diameter, subspherical, 85–102 μ, with a 3–4 μ thick wall; collapsing and becoming cup-shaped or wrinkled after drying out. Spec. in herb. Purdue Univ., Lafayette.

On *Potentilla canadensis*, Richmond, Va., U.S.A.

So far only 12 sporangial galls and sori have been found, and the dimensions given above relate to these. Obviously, the above diagnosis is based on meager information and will have to be modified when more material is collected and studied. For this reason it is not worthwhile to

give this species a specific name at present. No evidence of a prosorus was found in the stained sections, but I do not think this point is definitely settled. Possibly, the initial cell may be found to function as a prosorus when more material is studied. I had hoped to collect more material in the type locality, but I despair of doing so now because this locality has been cleared and leveled for a housing development. Accordingly, I am presenting this description now to direct attention to the occurrence of a long-cycled, simply dihomeogallic species on *P. canadensis*, which might be mistaken for *S. potentillae*.

So far only 6 long-cycled, simply dihomeogallic species have been described, including *S. amsinckiae* McMurphy, *S. maculans* Lingappa, and *S. namae* Karling of the subgenus *Microsynchytrium* and *S. papillatum* Farlow, *S. trichophilum* Correns & Tobler and *S. eremocarpae* Karling of the subgenus *Synchytrium* (*Eusynchytrium*). In the first three of these species the initial cell functions as a prosorus, while in the other three it is transformed directly into a sorus of sporangia. Insofar as it is known the present fungus seems to be more closely related to the latter three by the lack of a prosorus, but this remains to be determined by further study. Possibly, it may prove to be related to or identical with the one Crutchlow (1932) found on *P. fragariastrum* in North Wales. He reported the presence of summer sori which distinguishes it from *S. potentillae*.

SUMMARY

Synchytrium pilificum was found for the first time in America as a parasite of *Potentilla simplex* at Pennington, N. J. It infects the leaves, petioles, fruits and runners of the host and may lead to malformation of these organs and stunting of the plants. The galls which it induces bear from 50 to 165 hairs at their apices and are distinctive and characteristic in appearance. The fungus develops only thick-walled resting spores, and belongs, accordingly, in the subgenus *Pycnochytrium*. These spores germinate in the late winter, spring and summer, and function as prosori. Their contents grow out to form superficially attached sori of sporangia which give rise to planospores.

Another simply dihomeogallic *Synchytrium* on *Potentilla canadensis* was found at Richmond, Va., which is long-cycled and forms sporangial sori and sporangia in addition to resting spores. Inasmuch as the full life cycle is unknown, it is not identifiable at present.

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NOTES ON BOLETES. XIII

ESTHER A. DICK AND WALTER H. SNELL

This number reports upon a variety of topics and of boleti from east to west, with only a portion of the studies accomplished with the aid of National Science Foundation grant G-9043.

SOME ANOMALOUS ASSOCIATIONS

On a previous occasion (**5**, p. 375–377), we noted the very unusual occurrence of *Suillus granulatus* (L. ex Fr.) Kuntze subsp. *Snellii* Singer and *S. subluteus* (Pk.) Snell *apud* Slipp & Snell growing in pure stands of *Pinus rigida* on Cape Cod, Mass., in very rainy weather. We have a similar report with regard to the former species, except that the weather conditions had been only moderately wet instead of unusually rainy. The situation was a grassy roadside, again on Cape Cod in North Falmouth not far from Old Silver Beach in September, 1957. The trees up the bank and making up the woods were pitch pines 15–25 years old with a sprinkling of oaks, except for one interruption of 100 yards or more, which was only oaks except for one 25-year-old white pine in about the middle. Over a distance of a half-mile of this grassy roadside were found a half-dozen boleti that one would expect in association with the oaks and pitch pines, but the noteworthy occurrence was 100 or more carpophores of *S. granulatus* subsp. *Snellii*. A few of the specimens were in their expected place near the single white pine, and the remaining scores were rather evenly distributed along the two stretches of pitch pine entirely devoid of white pine.

In September, 1959, at Bretton Woods, New Hampshire, the first author found the same subspecies under spruce. The stand was young, thick with seedlings and young trees up to 5 feet high, with no balsam firs as far as observed and without a single white pine.

We find it difficult to explain the first of these situations. Most of the fruit bodies were far from the only white pine and it is very doubtful that white pines ever grew in quantity in this spot. White pine probably never grew in stands of more than a few trees anywhere on Cape Cod, at least east of Buzzard's Bay.

Suillus granulatus subsp. *Snellii* in the stand of young spruce is more easily explained if we make a guess—namely, that the area was originally

occupied by white pine, and upon the disappearance or removal of the white pine, was taken over entirely by the spruce. As to how this bolete lives without its normal, living white-pine associate—whether upon roots not yet dead, upon dead roots or in soil still favorable because of the influence or activity of the formerly living roots—is only another problem somewhat more abstruse than how the bolete spores germinate and the germ-mycelium nourishes itself and sets up its ultimate mycorrhizal relationship. It may be recalled that in a recent article (**8**, p. 568), there was mention of Singer's observations of *S. subaureus* (Pk.) Snell in pure stands of *Populus tremuloides* with or without white pine with which it is commonly associated in the northeast.

From time to time we have remarked upon the various host associations of *Leccinum aurantiacum* (Bull. ex S. F. Gray) S. F. Gray (**6**, p. 308, 309; **7**, p. 63). In northeastern North America, this species occurs commonly under *Populus* spp., but we have found the pine form not uncommonly near *Pinus rigida* and bearberry (*Arctostaphylos uva-ursi*)—under each or both—on Long Island, N. Y., and Nantucket, Mass., in Rhode Island, and on Cape Cod, Mass. In 1958, in a large close stand of mature spruce and balsam fir, with only an occasional hardwood, outside of Ste. Anne de la Pocatière, Quebec, *L. aurantiacum* was collected under *Populus tremuloides* in a few places but one collection was made in the spruce and balsam where there was not another aspen visible within 100 yards at least, and the only other tree was a small birch six feet high 25–30 feet away.

XEROCOMUS CONIFERARUM

Upon rare occasions over the years, we have collected single specimens of what we have called *X. spadiceus* (Fr. sensu Quél.) Quél.—something under conifers very much like *X. subtomentosus* (L. ex Fr.) Quél. but with pileus reddish-brown to chestnut, etc., and the stipe densely and minutely reddish- to reddish-brown-furfuraceous. Singer proposed the name *X. coniferarum* for this species (**4**, p. 297).

More recently we have now and then been finding what appeared to be this bolete under spruce and balsam fir in the Province of Quebec, and in 1959 gave our collections a little more attention. These specimens agreed closely with our former descriptions and with those of European mycologists. For the first time, some of the simple chemical tests were applied and it was found that the pileus reacted with a deep blue-green color to NH_4OH, one of the characteristics of Singer's Section Pseudophyllopori. This response on very moist specimens was very rapid and then the color disappeared almost as rapidly.

Other chemical reactions were found to be as follows: NH_4OH—on the flesh negative, on the tubes and pores somewhat dark to brownish; KOH—on the pileus and flesh negative, on the tubes a dark mahogany; $FeSO_4$—on pileus a deep green, on flesh and tubes a deep blue-green.

SUILLUS COTHURNATUS

In 1935, while engaged in some rust studies in a jack pine stand at Schuyler Falls, N. Y., one of us collected a young specimen that at the time was identified and labelled as *Suillus luteus* (L. ex Fr.) S. F. Gray, but with a notation that no annulus was present, perhaps because of the moist weather. The complete veil in this young stage was attached to the margin of the pileus and hanging in a curtain-like or bag-like manner, at its base loose and distant from the stipe, as it still is in the dried condition.

Recently, in a brief consideration of *S. luteus* and its relatives and their host-associates, we were struck by the unusual appearance of this specimen and its appended note, and we compared it with carpophores of *S. cothurnatus,* described by Singer (**4**, p. 261–265) as associated with certain hard pines in Florida and later found by him to be abundant in northern Michigan under jack pine. There appears to be no question that our specimen from New York is *S. cothurnatus.*

A NEW SUILLUS FROM UTAH

Suillus umbonatus sp. nov.

Pileo convexo, umbonato, sicco collecto, procul dubio viscido humido, glabro, "Olive Buff," glutine ferrugineo-brunneo. Carne "Cartridge Buff," dilute punicea fracta. Hymenophoro adnato, "Wax Yellow," brunneolo contuso, tubulis porisque glandulis adornatis, poris plus minusve boletinoideis, parvis. Stipite subcylindrico, apice ruguloso, glandulis dense adornato, annulato, subconcolori, basi plus minusve ferrugineo-brunneo. Sporis subellipsoideis, hyalinis, 7–10 × 3–4 μ. Apud sphagnum et alios muscos sub *Pino contorta* et *Vaccinio.* Utah. U. S. A.

Pileus broadly convex, with a rather acute umbo, 3.5–6 cm broad. *Surface* dry when collected, almost certainly viscid when wet, for the most part glabrous, when dry with scattered streaks and patches of dried gluten especially toward the margin, Olive Buff, with the dried gluten rusty-brown. *Flesh* Cartridge Buff becoming tinged faintly pinkish when cut. *Hymenophore* adnate, Wax Yellow, becoming brownish where bruised, tube-walls rather thickly and finely reddish-brown-glandular-dotted; pores somewhat radially oriented, subangular, .5–1 mm broad, sparsely black-glandular-dotted when dry. *Stipe* subcylin-

dric, even except for the apex sparsely rugose from the descending walls of the tubes, rather thickly reddish-brown-glandular-dotted to the base, annulate, subconcolorous, more or less blotched or streaked reddish-brown at the base, the annulus brown to pinkish-brown and when dried forming a conspicuous zone. *Spores* ellipsoid or subellipsoid, hyaline, ranging from 7–10 × 3–4 μ but the great majority remarkably uniform in size, 8–9 × 3.5 μ.

Amongst sphagnum and other mosses under *Pinus contorta* and *Vaccinium*. Trial Lake Campground, Uintah Mts., Summit County, Utah. Coll. *K. H. McKnight*. TYPE in WHS Bolete Herbarium, no. 2281.

In the dried condition, this looks remarkably like *Boletinus decipiens* (Berk. & Curt.) Pk., differing in the umbo, the viscid surface and lack of scales, the glandular-dotted stipe and tubes, and the persistent annulus. Apparently it belongs in the Subsection Angustiporini of the Section Granulati.

A FEW BOLETI FROM LOUISIANA

Among a few collections from Louisiana sent in by Bernard Lowy, there were several that showed a southwestern extension of range and one species apparently new. We are quite certain of the identifications of the following: *Phlebopus sulfureus* (Fr.) Singer, *Pulveroboletus auriporus* (Pk.) Singer, *Tylopilus Ballouii* (Pk.) Singer, and *T. plumbeo-violaceus* (Snell & Dick) Snell & Dick. We are reasonably certain concerning *Leccinum rubropunctum* (Pk.) Singer. Another collection appears to be *Xanthoconium affine* (Pk.) Singer, but no spore-print was available. One other collection of carpophores 6–15 cm broad appears to be *Boletus rubellus* Krombh. subsp. *bicolor* (Pk.) Singer, but in view of the lack of notes on color changes and of our unfamiliarity with some of Singer's subspecies and forms of the species, we are reluctant to make a definite identification.

What appears to be a new species follows—questionably in the Section Fellei.

Tylopilus subunicolor sp. nov.

Pileo subpulvinato vel subapplanato, plus minusve rimoso, glabro, fortasse in margine coactato, ochraceo-fulvo vel ochraceo-brunneo, rimis luteolis, 5–6 cm lato. Carne dilute ochracea, fortasse ad marginem luteore, immutanti. Hymenophoro adnato, primum albo, mox dilute roseotincto, deinde dilute ochraceo-brunneo, fortasse aliubi atque aliubi roseotincto, poris concoloratis, parvis. Stipite subaequali, minute furfuraceo, infra fortasse subglabro, basi fortasse subtomentoso, concolorato vel pallidiore, 2–5 cm × 7–15 mm. Sporis in cumulo "Cinnamon Brown" vel

"Sayal Brown," subellipsoideis, hyalinis, 8–11 × 3.5–4 μ. Mycelio e subluteo ad ochraceum.

In quercetis. Louisiana, U. S. A.

Pileus subpulvinate to subapplanate, 5–6 cm broad. *Surface* more or less rimose, glabrous, perhaps matted-tomentose on the margin; ochraceous-tawny to ochraceous-brown, yellowish in the cracks. *Flesh* pale ochraceous, perhaps more yellow towards the margin, unchanging; odor when re-wet slightly of celery. *Hymenophore* adnate, at first white, soon with a rosy tint, then light ochraceous-brown, perhaps with a rosy tint in places; tubes 3–4 mm long; pores concolorous, 1 mm or less broad. *Stipe* subequal, perhaps expanded at apex, minutely furfuraceous, more so at apex, less so towards the base, lower half possibly subglabrous, base perhaps tomentose; concolorous with surface of pileus or perhaps paler; within as in pileus; 2–5 cm long, 7–15 mm thick except enlarged apex. *Spores* in deposit Cinnamon Brown where thick, Sayal Brown where thinner, subellipsoid, hyaline, 8–11 × 3.5–4 μ, mostly 9–10 × 3.5–3.5+ μ. *Mycelium* yellowish to ochraceous. KOH on dried flesh—more orange.

Under oaks. Louisiana, U. S. A.

TYPE collection, WHS Bolete Herbarium, no. 2273, Brown University, Providence, R. I.; portion of original collection, *B. Lowy* no. 7795, Louisiana State University, Baton Rouge, La.

NEW SPECIES FROM CALIFORNIA

Most of a number of collections sent by Wm. Bridge Cooke apparently are new species but, except in one case, before describing these as new, we prefer to await more material. The most distinctive feature of this one is its dull colors. It belongs in the Section Subtomentosi.

Xerocomus lenticolor sp. nov.

Pileo e plano ad depressum, ad marginem rimoso, generaliter tomentoso, centraliter e floccoso-tomentoso ad floccoso-pilosum, ad partem exteriorem vario, sordide luteo-brunneo vel griseo-brunneo, rimis e griseis ad aurantiacas, 3–6 cm lato. Carne probabiliter subalba, ad marginem cyanescenti. Hymenophoro adnato, luteo, cyanescenti, poris ad 1 mm latis. Stipite crasso, basi subbulboso, e subglabro ad minutissime brunneo-punctulatum, apice dilute luteo, infra e sordido ad brunneolum, basi aurantiaco, intra cyanescenti ad exteriorem sed non in basi aurantiaca, 20–35 × 12–25 mm. Sporis in cumulo probabiliter olivaceis, ellipsoideo-subfusiformibus, dilutissime subviridibus, 8–11 × 3–3.5 μ.

Sub arboribus coniferis mixtis. California, U. S. A.

Pileus plane to depressed, up to 6 cm broad. *Surface* rimose towards the margin, in general tomentose, the center tufted-tomentose to tufted-pilose with various gradations outward, dingy yellowish-brown or gray-

ish-brown, the cracks gray to orange. *Flesh* probably whitish, turning blue towards the margin. *Hymenophore* adnate, yellow, changing to blue; pores up to 1 mm broad. *Stipe* thick, enlarged to subbulbous at the base, subglabrous to very finely brown-punctate, light yellow at the apex, more dingy to brownish downward, orange at the base; within concolorous with the flesh of the pileus, orange at the base, turning blue toward the outside except at the orange base; 2–3.5 cm long, 12–25 mm thick. *Spores* of unknown color in deposit but without doubt olivaceous, elliptic-subfusiform, very pale greenish, 8–11 × 3–3.5 μ, a few 12 × 4 μ, mostly 9–10 × 3.5 μ.

Under mixed conifers, mainly *Pinus Jeffreyi, Libocedrus decurrens,* and *Abies concolor,* nearest the last. Bear Springs, Mt. Shasta, Siskiyou Co., California. Elevation 5000 feet.

TYPE collection, WHS Bolete Herbarium no. 2277, Brown University, Providence, R. I.—also, *Wm. B. Cooke* no. 31,197, Cincinnati, Ohio.

For some years we heard from members of the Mycological Society of San Francisco about a black bolete in California, but no one was able to obtain specimens for our edification and study. Late in February of 1959, however, Miss Esther Whited sent us a collection from under Bishop pine at Inverness, California, that arrived in a reasonably fresh and good condition. This species appears to be a *Porphyrellus* rather than a *Tylopilus* because of the reactions to chemicals, the color of the spore-print, the shape, size, color, thickness of the wall, the papilla and the apparent germ-pore of the spores, and the rather large basidia and cystidia. It apparently belongs in Singer's Section Tristes even though it does have a coarsely reticulate stipe.

There is some question about the colors and succession of colors of the pores as noted by the collector and as seen by us in the still fresh condition after four days in the mail. Therefore, some correction may be necessary when the species is collected again.

Porphyrellus atrofuscus sp. nov.

Pileo e hemispherico ad convexum, velutino vel subvelutino, fuliginoso-olivaceo-atro, ad 8.5 cm lato. Carne albida, grisea vel brunneogrisea insecta, passim rubro-brunnea vel nigridia. Hymenophoro e depresso ad plus minusve liberum, primo albo vel albido, deinde ochraceo, nigricanti insecto; poris primo nigridiis (?), deinde hinnuleorum colore rubrotincto et postremo ochraceis, parvis. Stipite crassiore, plerumque curvato, sursum coartanti, minute et dense e furfuraceo vel subpiloso ad glabrum, sursum forte reticulato et nigridiobrunneo, deorsum striato et pallido vel albido, intra cum pilei carne concolorato, ad 10 cm × 2.5–3.5 cm. Sporis in cumulo "Bay," "Vandyke Brown" vel "Chestnut Brown," subellipsoideis vel oblongo-ellipsoideis, subpapillatis, dilute brunneis, 11–17(19) × 4.5–6.5(7) μ.

Sub *Pino muricata,* Inverness, California, U. S. A.

Pileus convex, up to 8.5 cm broad. *Surface* velutinous or subvelutinous, sooty-olivaceous-black. *Flesh* whitish, darkening on exposure to grayish or brownish-gray, with reddish-brown or blackish areas; odor and taste not noteworthy. *Hymenophore* depressed to more or less free, at first white or whitish becoming Honey Yellow or ochraceous, blackening upon exposure; tubes up to 15 mm long; pores whitish at first (?) but, as reported, blackish at first becoming a reddish-fawn color (from the spores?), later found to be ochraceous or Honey Yellow to Orange Citrine, Antique Brown in places, blackish when dried, small to medium; in obtaining a spore-print, white paper stained a bright cerulean blue which later became blue-green. *Stipe* rather thick, usually more or less curved, tapering upward; minutely and closely furfuraceous or subpilose to glabrous, reticulate one-third to two-thirds of the length, with the reticulum especially above very narrow, elongated, and much raised to give almost a striate appearance, with raised striations below; blackish-brown above, whitish to dingy below, with the striations dark brown to blackish; within whitish, becoming grayish to brownish, with brown to black areas and streaks; up to 10 cm long, 2.5–3.5 cm thick. *Spores* in deposit between Bay, Vandyke Brown and Chestnut Brown, subellipsoid or oblong-ellipsoid, some somewhat narrowed at distal end, with a small papilla, with no or very little suprahilar depression, apparently with a narrow germ-pore, moderately thick-walled, light to dark watery-brown (perhaps between a dilute Isabella Color or Old Gold with daylight, when older or dried the central body a pale greenish and the outside reddish or pinkish), 11–17 × 4.5–6.5 μ, a few up to 19 × 7 μ, mostly 14–15 × 5.5–6 μ.

Under *Pinus muricata*, Inverness, California, U. S. A.
TYPE collection, WHS Bolete Herbarium no. 2269.

Chemical reactions

On surface of pileus and on tubes—no response with anything tried. On flesh of pileus and stipe: KOH—mahogany; NH_4—slightly yellowish; $FeSO_4$—very pale green or a dull gray-green.

Microscopical

Surface of pileus a compacted, eroded pile of pigmented, brown hyphae hardly encrusted but with some granular material which was not colored.

Cystidia, etc.

On colored pores—each one a stalk with a somewhat elongate, lemon-shaped tip which is pointed but not papillate, apparently hyaline but more or less encrusted with a brown pigment, the whole 60–65 × 14–17 μ, the long lemon-shaped tip 30–32 μ long; also clavate hyphal ends 40–52 × 8–10 μ.

On the tube walls—similar structures which for the most part had a small papilla at the tip of the swollen portion.
Basidia—clavate, 35–42 × 12–14 μ.

BOLETUS APPENDICULATUS (?) IN CALIFORNIA

In December, 1957, Salvatore Billeci of the Mycological Society of San Francisco sent a "yellow" bolete collected by others under oaks and manzanita near Grass Valley, California, elevation 2800 ft, that was most interesting and for a while quite bothersome. This species is reported as having increased enormously in that area in the last 10 years. The fruit-bodies grow very large, up to 30 cm broad and 10 cm thick, with stipes only 7–9 cm long but up to 10 or 12 cm thick, and may weigh 5–7 pounds. They are so firm and solid as well as weighty that the original collector hesitates to kick them around because that form of diversion is painful.

Information upon the usefulness of the species for the table is variable. Some have reported distress after eating these fruit-bodies, especially when "freshly cooked" (cooked fresh, or freshly cooked after drying?). Others report a good flavor and no distress, whether or not the water in which they have been cooked was discarded.

This "yellow" species is sufficiently yellow to justify this field appellation but the surface of the pileus has overtones of tan or pale brownish with darker areas but still more yellowish than brownish. The carpophores turn blue without and within when bruised or cut. The surface is glabrous for the most part but innately and very minutely fibrillose in places. The stipe is somewhat reticulate, and minutely and more or less obscurely furfuraceous or somewhat fibrillose in places. Everyone has agreed—and repeatedly upon inquiry—that the taste is not bitter, and therefore the species is not *B. radicans* Pers. ex Fr. Also, it does not appear to be *B. luridellus* (Murr.) Murr. of Florida, one of the Luridi, because of much lighter colors of the pileus, lack of red at the base of the stipe, size of spores, and other characters.

The only species with which this collection shows close agreement is the European *B. appendiculatus* Schaeff. ex Fr. Our specimens have no reddish-brown colors or tinges on the pileus that are mentioned in the descriptions as occasional, but Kallenbach's plate 31 (2) shows hardly any of this and in any event a footnote on page 84 warns that the entire plate is too reddish-brown. Our specimens are somewhat innately fibrillose under a binocular microscope or subtomentose and fibrillose in places only and not so conspicuously so as described for

B. appendiculatus, although plates of this species show many pilei without this clothing and European specimens in our posession do not have much of it. The California material shows no changes to rusty-brown when bruised, but turns blue. The flesh turns more blue than described for *B. appendiculatus* but apparently just as slowly.

The reticulation of the stipes of our specimens extends only about 1–3 cm but the European figures show varying amounts and the descriptions all mention that the reticulation almost disappears in age. Our stipes show a slight amount of indefinite and obscure furfuration, which is not mentioned in any descriptions of which we know, although the European *B. appendiculatus* in our possession does have the same sort and amount of mealiness on the stipe below the reticulation. Further, our stipes show no radication, but while the European descriptions all mention it, most of the illustrations and our European specimens show no pronounced radication.

The spores agree well with the descriptions of the spores of *B. appendiculatus,* perhaps narrower by .5 μ, and they differ only slightly in shape from those of the European material we have, which incidentally is rather young.

The differences pointed out in the foregoing discussion appear to be of insufficient magnitude or importance to justify the description of a new species, and therefore, for the time being at least, we are considering the California collection to be *B. appendiculatus.*

A NEW LECCINUM

From time to time in the past 30 years in New England and New York, we have occasionally come across a *Leccinum* which was very dark to almost black, had a tomentose to bunchy-tomentose surface and usually became darker inside when cut open. For herbarium purposes, we have been tentatively calling this *"L. leucophaeum* (Pers.)." There is no point in again reviewing the extensive literature upon Persoon's species, but suffice it to say that the epithet *"leucophaeus"* has been much misunderstood, it has been variously applied, it has been considered to be a synonym of *scaber* by various French and German mycologists, and has been accepted by others as a good species (e.g., Gilbert, **1**, p. 184–185). Singer (**3**, p. 190) concluded that it is impossible to identify Persoon's species with certainty and that *"leucophaeus"* is a *nomen dubium* which should be abandoned. Therefore, whether or not we have been finding what is Persoon's *leucophaeus,* our collections are sufficiently different from the Leccinums we know to deserve a name.

This species very much resembles the darker forms of *L. oxydabile* (Sing.) Sing. It differs in three respects. The covering layer of the pileus is a trichoderm of somewhat thinner hyphal ends with few if any catenulate or detached, short, round-ended cells or units and no sphaerocysts as far as observed. The flesh of pileus and stipe becomes dingy or grayish to blackish instead of pinkish or more or less reddish. Also, thus far we have observed no blue-green at the base of the stipe.

Leccinum subleucophaeum sp. nov.

Pileo sicco, fortasse plus minusve viscido humido, e tomentoso ad fasciculato-tomentosum, punctuloso-tomentoso vel minute reticulato-cristato, raro in tempestate pluvia paene glabro, fusco, nigridio vel nigro, epicute sine cellulis rotundis. Carne alba, subgrisea vel fusciore fracta. Hymenophoro e adnato ad liberum, plus minusve depresso, primo albo, deinde subgriseo, poris rotundis, minutis. Stipite sursum coartanti, subbulboso, subrugoso vel pseudoreticulato, e furfuraceo-punctato ad scabrum, e albido ad subgriseum, asperitatibus superne albidis et deorsum subfuscis vel nigridiis; intra pileo simili, basi plus minusve ochraceo; 7–10 cm × 11–25 mm. Sporis in cumulo "Snuff Brown," fusiformi-ellipsoideis, 14–22 × 5–7 μ. Sub *Betula*.

Pileus pulvinate, 5–9 cm broad. *Surface* dry, perhaps becoming more or less viscid when wet, tomentose to bunchy-tomentose, punctulose-tomentose, minutely reticulate-cristate or reticulate-fibrillose, rarely almost glabrous in wet weather, dark brown to black or blackish, often blacker on the disc, the cuticle a trichoderm with few if any short, round-ended cells and no sphaerocysts. *Flesh* firm, white, becoming grayish or perhaps quite dark when old; odor and taste mild to insignificant. *Hymenophore* adnate or somewhat depressed to nearly free, at first white, becoming grayish; pores rotund, minute, 2 to a mm. *Stipe* firm, tapering upward, more or less bulbous, more or less irregularly rugose or pseudoreticulate, sharply rugose at the apex when young, crowded-furfuraceous-punctate to scabrous; whitish to grayish, darkened by the crowded dots and scabrosity which are whitish or grayish above and brownish or blackish below; within fibrous, white to dingy, becoming more dingy, more or less ochraceous at the base; 7–10 cm long, 11–25 mm thick. *Spores* Snuff Brown in deposit, fusiform-ellipsoid or naviculate, olivaceous-brown, 13–22 × 5–7 μ, a few broader, mostly 16–19 × 5.5–6 μ. *Mycelium* white.

Under birches. Rare. New England and New York.

TYPE collection, WHS Bolete Herbarium no. 210.

Microscopical

The cuticle of the pileus a trichoderm of more or less erect hyphal ends which makes a tomentum at first and then becomes more or less flattened in areas between minute clumps, punctulations or

a minute network of erect hyphae, with the exterior surfaces of these superficial features matted with spores; the component hyphae or hyphal ends regular, septate, hyaline or brown, mostly 7–11 μ in diameter, a few up to 18 μ, very rarely if at all forming short, swollen or round-ended cells or units in chains and never forming sphaerocysts as far as observed.

Cystidia

Quite abundant at times and not found at others, fusoid or ventricose-fusoid with mucronate or ampullaceous apex, hyaline, 40–50 × 9–11 μ.

Basidia

Clavate or subclavate, 25–28 × 9–11 μ.

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PECTOLYTIC ENZYMES PRODUCED BY BOTRYOSPHAERIA RIBIS AND PENICILLIUM ITALICUM [1]

RALPH P. COLLINS AND WILLIAM F. SLEDJESKI [2]

(WITH 3 FIGURES)

INTRODUCTION

We are studying the production of pectin methylesterase (PME), pectin polygalacturonase (PG), and pectin depolymerase (DP) by soft rot organisms and report here pectolytic enzymes produced by *Botryosphaeria ribis* Gross & Dug. and *Penicillium italicum* Wehmer.

There have been several reports concerning the apple fruit rot caused by *B. ribis* (**4**, **6**, **8**). Two types of fruit rot have been reported. Fenner (**2**) noted lesions similar to those of bitter rot. These lesions are circular and slightly sunken and the tissue becomes mushy. The second type is a post-harvest rot characterized by the rapid breakdown of tissue; the tissues may undergo dissolution even with no external evidence of the incitant.

P. italicum typically produces a soft "blue mold rot" on citrus fruits. Infected fruits quickly lose their shape and may deteriorate to a watery mass.

The physiology of these organisms has not been extensively investigated but Dingle and Solomons (**1**), using a cup-plate assay technique, have shown that *P. italicum* produces pectin polygalacturonase and Sitterly and Shay (**7**) have shown that critical time for fruit infection by *B. ribis* apparently is connected with the onset of the climacteric rise in respiration. McClendon et al. (**5**) have shown that enzyme preparations from apples rotted by *B. ribis* hydrolyzed galacturan, galactan, araban, galactomannan, xylan, and pectic acid groups but not cellulose. They did not test the pectolytic activity of culture filtrates of *B. ribis*.

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[2] Recipient of National Science Foundation undergraduate trainee grant, summer of 1959. The writers are grateful to Mr. Chester R. Roistacher for supplying a culture of *P. italicum* and to Dr. E. S. Luttrell for supplying a culture of *B. ribis*.

TABLE I

PECTIN METHYLESTERASE ACTIVITY OF WHEAT BRAN CULTURE FILTRATES

Organism	Culture filtrate	PME*
Botryosphaeria ribis	bran	0.827
Penicillium italicum	bran	1.08

* mg methoxyl removed per ml filtrate.

MATERIALS AND METHODS

The organisms were grown in bottles containing 25 g of bran moistened with 100 ml of water. The medium was autoclaved at 15 psi for 20 minutes. The inoculum was grown in 250 ml shaker flasks containing 80 ml of potato dextrose broth. When growth was good, the inoculum was macerated in a Waring blender; 15 to 20 ml were pipetted into bottles containing the bran and water and incubated at 25–1° C.

Pectin methylesterase (PME) activity was determined by the method of Winstead and Walker (9). Activity was determined by measuring

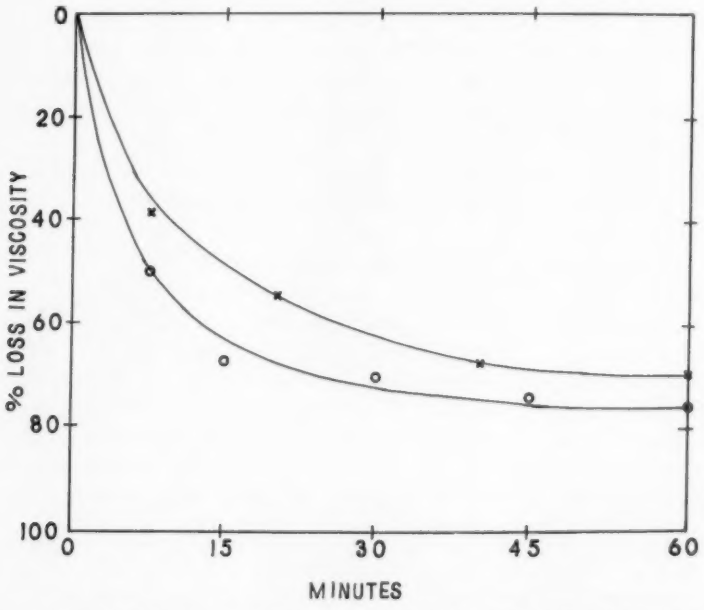

FIG. 1. Loss in viscosity of a 1.5% pectin solution (x) and a 1% sodium polypectate solution (o) caused by culture filtrates of *Botryosphaeria ribis*.

the increase in reducing groups and the loss in viscosity of the substrate. Increase in reducing groups was measured by the method of Jansen and MacDonnell (3). Viscosity changes were determined by means of Ostwald Fenske viscosimeters held in a 30° C water bath. A 1% solution of sodium polypectate or citrus pectin (California Fruit Growers Exchange) or citrus pectin containing citrate buffer at pH 4.0 was used as substrate. Paper chromatograms were run to test for monogalacturonic acid, formed as the result of polygalacturonase activity on pectic acids. The solvent system used was a 4:1:5 mixture of butanol, acetic acid, and water. The chromatograms were developed using a benzidine solution (0.5 g benzidine, 10 ml acetic acid, 10 ml of 45% trichloracetic acid, and 100 ml of 95% ethanol). Monogalacturonic acid was indicated by a pink spot after the sprayed papers were heated in a 105° C oven for 2 minutes.

RESULTS

Culture filtrates of *B. ribis* and *P. italicum* obtained from a bran medium gave a positive test for PME. The PME activity was deter-

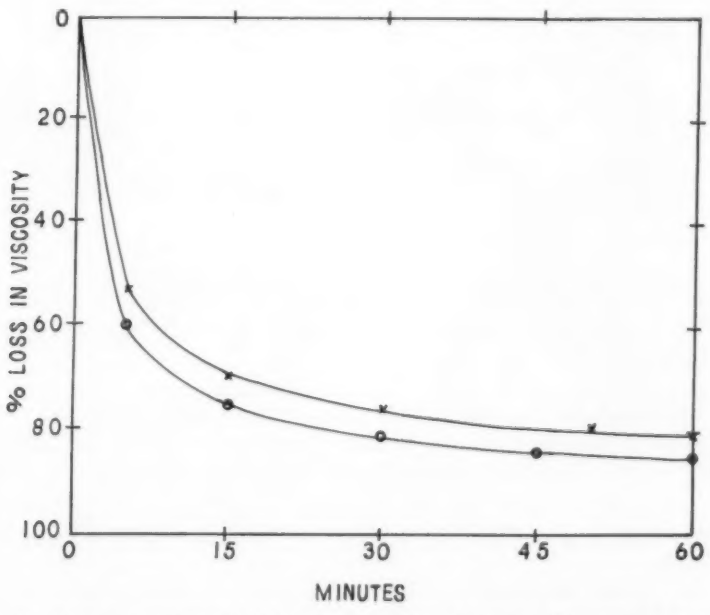

FIG. 2. Loss in viscosity of a 1.5% pectin solution (x) and a 1% sodium polypectate solution (o) caused by culture filtrates of *Penicillium italicum*.

mined by measuring the mg of methoxyl groups hydrolized from a 1% pectin solution by the enzyme present in 1 ml of culture filtrate in 3 hours (TABLE I).

Filtrates of *B. ribis* from the bran medium showed PG activity. The pectin-splitting activity of the filtrates was determined by the decrease in viscosity of a 1.5% pectin solution and a 1.0% sodium polypectate solution. The activity was higher when the substrate was sodium polypectate (FIG. 1). Filtrates of *P. italicum* also showed strong PG activ-

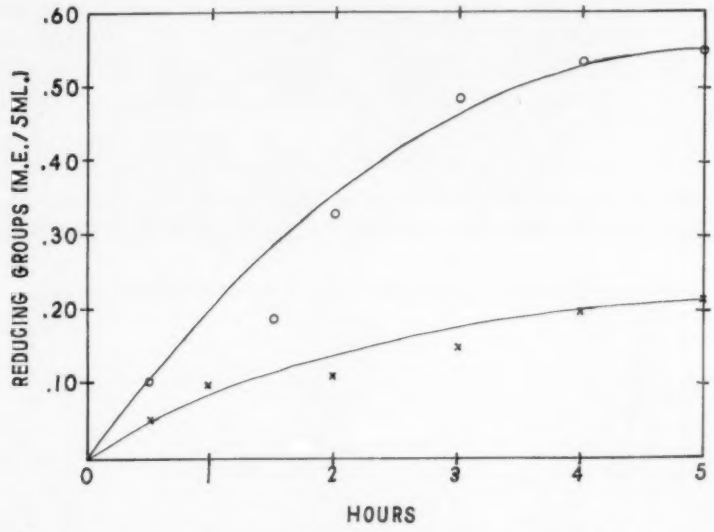

FIG. 3. Milliequivalents (meq.) of reducing groups produced by reaction of culture filtrates with a 0.5% sodium polypectate. (o) meq. of reducing groups produced by *Botryosphaeria ribis* and (x) meq. of reducing groups produced by *Penicillium italicum*.

ity when added to sodium polypectate or citrus pectin solutions. Filtrates of *P. italicum* also showed more activity when sodium polypectate was the substrate but the difference was less with *B. ribis* (FIG. 2). An increase in reducing groups was found in both *B. ribis* and *P. italicum* when 10 ml of each filtrate was added to 90 ml of sodium polypectate (FIG. 3).

To test for D-galacturonic acid, samples from the filtrates were applied to Whatman No. 1 strips and the strips were developed using descending chromatographic techniques. The controls were a heat in-

activated sample and a sample of D-α-galacturonic acid. After 12 hr of incubation significant amounts of galacturonic acid were detected in culture filtrates of *B. ribis* and *P. italicum*.

DISCUSSION

Botryosphaeria ribis and *Penicillium italicum* showed PG and PME activity but no DP activity. Apple fruits infected with *B. ribis* and citrus fruits infected with *P. italicum* are characterized by disorganization of affected tissues. The action of pectic enzymes on the cell wall could explain the characteristic softening of tissues infected by *B. ribis* or *P. italicum*.

Experiments to test the macerating activity of *B. ribis* and *P. italicum* were not performed but would be desirable. The observation made by Dingle and Solomons (1) concerning the production of pectin polygalacturonase by *P. italicum* has been confirmed. We have shown that PME is produced.

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RELATIONSHIPS OF THE ACTINOMYCETALES [1]

C. W. Hesseltine

(with 1 figure)

The structure of Actinomyces certainly favours the view that it is of the nature of a Fungus, but it has no closer resemblance than this to well-known Fungi. It is not possible therefore to assign it a place in the system, or to form any clear idea of the history of its growth and development from the analogy of other Fungi.—De Bary, 1887.

Numerous papers, books and reviews within the last 15 years have summarized many aspects of the Actinomycetales. Members of this order have been exploited more fully than any other group for the production of antibiotics and related compounds. As a consequence of the commercial exploitation of the actinomycetes, classification of species and the description of new forms have been vigorously attacked. Numerous investigators in the United States, Germany, Switzerland, Italy, Russia and Japan have gradually come to agreement on characteristics that are the most stable, and hence fundamental, in the characterization of species, especially in the genus *Streptomyces*. Morphology of the aerial fructifications of the Actinomycetales is stable on a variety of media where growth approaches the typical growth in nature. In addition, characteristics such as mass spore color and even pigmentation of the substrate mycelium are of considerable importance in the separation of groups of species. This is not particularly surprising since even among bacteria the basic means of separation of genera is based on morphology. The characteristics most useful in separating species within a morphological and spore color group are being very actively studied in many laboratories, and already some of the characteristics are becoming clear. This paper does not propose to discuss these matters further. On the contrary, an evaluation of the knowledge as it exists today regarding the relationship of actinomycetes to other orders and classes of microorganisms has not been thoroughly weighed, or, if so, it was con-

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cerned only with a single characteristic such as the relationship derived from a comparison of the cell wall composition of actinomycetes with cell wall composition of other microorganisms. Various views have been expressed regarding the proper placement of the actinomycetes within the bacteria or fungi or regarding them as an independent evolutionary line not closely related to either. The available facts, therefore, concerning the Actinomycetales, and their possible relationships to other groups, are evaluated here. Besides being of academic interest, such an evaluation has considerable practical significance. It is important to decide whether the actinomycetes should be grouped with the bacteria or fungi since one must choose either the Code of Bacterial Nomenclature or the Code of Botanical Nomenclature. Should a new taxon in this order be described with a Latin description, or not?

One is handicapped by the absence of fossil records and by the complex developmental morphology encountered in higher fungi and flowering plants. In fact, the occurrence of a sexual process has not been conclusively demonstrated in the Actinomycetales.

The order Actinomycetales as it is treated in the 7th edition of Bergey's Manual of Determinative Bacteriology contains the following families and genera:

Family Mycobacteriaceae
Mycobacterium
Mycococcus
Family Actinomycetaceae
Nocardia
Actinomyces
Family Streptomycetaceae
Streptomyces
Micromonospora
Thermoactinomyces
Family Actinoplanaceae
Actinoplanes
Streptosporangium

Whether all of these genera are legitimate is still in some doubt. Thus Meyen (**1827**) described a new genus *Actinomyce* and a single species, *A. horkelii,* many years before Harz coined the name *Actinomyces.* In correspondence with G. W. Martin he states that *Actinomyce* Meyen 1827 and *Actinomyces* Harz 1879 are orthographical variants and hence Harz's name is a later homonym. Μύκης is singular and therefore *Actinomyce* should be corrected to *Actinomyces.* Undoubtedly, older genera

not listed above belong in this group of microorganisms. For example, *Dermatophilus* van Saceghem (syn. *Polysepta* Thompson and Bisset) is certainly a member of this order and, in fact, Austwick (**1958**) described a new family Dermatophilaceae containing the genus *Dermatophilus* and added it to the actinomycetes.

MORPHOLOGICAL EVIDENCE

Several opinions have been expressed as to the origin of the Actinomycetales. A number of authors, including Drechsler (**1919**), held the view that *Actinomyces* (*Streptomyces*) should be grouped with the Fungi Imperfecti. This contention was based upon the production of profuse mycelium and chains of aerial conidia. A second view holds that actinomycetes are related to bacteria because of their size and chemical composition. According to this theory, *Streptomyces* terminated a phylogenetic line from the corynebacteria and mycobacteria. Another theory stated that actinomycetes are bacteria whose ancestorial forms degenerated to *Mycobacterium* and *Corynebacterium*. Another scheme postulated a separate origin of actinomycetes wherein there was no close relationship to either bacteria or fungi. Thus Krasilnikov (**1949**) proposed a classification in which Protophyta was divided into two groups, the chlorophyll-containing Schizophyceae and the achlorophyllous Schizomyceae. The latter group of organisms contained four classes with the exception of the fungi: Actinomycetes, Eubacteriae, Mycobacteriae and Spirochaetae. He stated that the actinomycetes (ray fungi) differed from molds in the absence of a nucleus.

Any review of the relationships of actinomycetes to other microorganisms is handicapped by the voluminous literature in a variety of journals; many of the reports are based on studies of pathogenic species. For a better understanding of the group, more attention must be paid to the saprophytic forms if one assumes that pathogens are not the ancestors of saprophytes. Consequently, studies on nonpathogenic organisms will be mainly considered; in particular, those organisms that are placed in *Streptomyces*, the commonest and most widely studied genus.

Drechsler (**1919**) was one of the earliest investigators of actinomycete morphology. The vegetative mycelium formed in the substrate is very small, never exceeding 1–2 μ in diameter. Except for its small size, the mycelium is similar to that of fungi. This similarity appears to be the case also in species of *Mycobacterium* (Spitznagel and Sharp, **1959**). Septations occur in actinomycetes. The formation of branches similar to that seen in fungi is encountered in all genera treated in the order by

Bergey's Manual of Determinative Bacteriology. Strangely, the colony appearance, manner of sporulation and oxygen requirements of actinomycetes have changed, but not the diameter of the vegetative mycelium. Fruiting stalks or sporophores are produced aerially from the vegetative mycelium. The sporophore divides in a variety of ways. The branches are converted into rows of spores with the whole fertile branch often somewhat larger than the nonfruiting portion of the sporophore. The whole sporophore is characteristic of each species. In some cases the branches are arranged in a wavy or flexuous fashion, others in open or tight spirals with as many as 20 turns. Each of the morphological forms of the sporophores (Hesseltine et al., **1954**) can be encountered in the fungi.

The manner in which sporulation occurs is still in doubt. Some have claimed sporulation begins apically and proceeds downward to the base of the branch. According to Drechsler (**1919**) the diameter of the aerial mycelium reaches its maximum size at the time it arises through the elongation of the growing filament tip. At their beginning the fertile branches arising from the sporophores are thinner than the nonfertile aerial hyphae. Later, however, when the fertile branches have nearly attained their final linear extension, they increase in diameter. In some forms where the aerial filaments are nearly simple, sporulation may occur almost to the substrate mycelium and is reminiscent of the condition reported in certain strains of *Nocardia*. Recent studies prove that sporulation in *Nocardia* occurs in the same fashion as that in *Streptomyces* (Bradley, **1959**). Furthermore, it appears that *Streptomyces* and *Nocardia* cannot be separated. Electron microscope studies recently have shown the spores of *Streptomyces* to have walls as distinctive as those encountered in the fungi. The surface of the spore may assume a smooth or an irregular outline, or may be echinulate or hairy. The spores range in shape from spherical, oval to cylindrical. Another interesting characteristic of *Streptomyces* spores is their occurrence in a linear series which remains intact in the air. Contrary to accepted belief, spore chains are not readily disrupted upon exposure to air movements. When Petri dishes with sporulated cultures are opened for examination, little or no aerial contamination of the work bench occurs. However, the addition of water to cultures with dry aerial sporophores markedly changes the situation, except in those species which have a verticillate manner of branching. Once the spore chains are moistened, they immediately fly apart with each spore apparently separating from its nearest neighbors in the chain. From personal observations it would not be surprising to find that the explosive disruption of spores on wet-

ting is an actual method of spore dispersal. After precipitation, either in the form of rain or dew, disruption of spores at the surface of the earth would aid in their dispersal to new locations where they could germinate and establish new colonies, provided the natural substrate was still moist.

Carvajal (**1947**) first noticed that several species of *Streptomyces*, including *S. griseus* (Krain.) Waks. & Henrici, formed spores in aerated submerged cultures just as do strains of *Penicillium*. Unlike bacteria and like most fungi, *Streptomyces* gave submerged growth in a mycelial form. The long chains of spores visible in the submerged mycelium later broke into individual elements and some, in turn, germinated in the same manner as do aerial spores.

Comparable to fungal spores, those of *Streptomyces* placed on suitable nutrient media first undergo appreciable swelling, germinate with one or more germ tubes, and in a few hours begin to form mycelium. The germination of spores to form a fungus-like mycelium is found in *Actinomyces* (Morris, **1951**), *Micromonospora* (Erikson, **1953**), *Waksmania* (Lechevalier and Lechevalier, **1957**) and *Nocardia* (McClung, **1949**). Furthermore, McGregor (**1954**) who studied nuclear division in spores prior to the formation of a germ tube, states that the method of nuclear division closely resembles mitosis in certain higher organisms and lower plants. It now appears certain that spores of actinomycetes have at least one nucleus.

The spores in bacteria are heat-resistant. In contrast spores in actinomycetes, like fungal spores, are readily heat-killed. The function of spores in actinomycetes is: (1) to increase the population and (2) to maintain the species during periods of adversity. Adverse conditions might include the absence of proper nutrients, insufficient warmth, or a lack of adequate moisture. On the other hand, the single spore in a bacterial sporangium does not function in multiplication of the species. I should also mention that cells of nearly all bacteria can be lyophilized successfully. In actinomycetes the mature spores and the cells of the substrate mycelium functioning as spores can be lyophilized but not the active vegetative cells. In bacteria a single organism is a cell which is entirely used to produce two new individuals, whereas a single *Streptomyces* organism has a more differentiated body. Only a part of the cytoplasm is used in the next generation while much of the cellular structure is foreordained to death. Hence a bacterial colony and an actinomycete colony are not at all comparable.

In some *Streptomyces* at least, the substrate mycelium forms oidia-like spores which differ from those formed aerially. Many years ago

Drechsler (**1919**), Neukirch (**1902**) and others reported these spore-like bodies. They resemble oidia seen in the Mucorales. Oidiospores form germ tubes in a similar fashion to the aerial spores.

Returning to the mode of sporulation in *Streptomyces,* one must conclude that it is not yet clear whether sporulation proceeds from the tip of a fertile branch towards its base or whether spores are formed simultaneously. The process may vary from species to species. However, it is important to realize that recently, since fruiting structures have been studied in the genus with the electron microscope, it has become apparent that the spores, in some forms at least, are formed within a membrane—a sporangial wall. Vernon (**1955**) found that spore formation in streptomycetes occurred within the hyphal wall. The cytoplasm divided into segments separated by more or less dense portions. The fragments were somewhat irregular and the divisions appeared to occur simultaneously. For a time the old hyphal wall encompassed the spores in chains. The sheath or sporangium either split open or disintegrated into small fragments to release the spores. This case was not unusual, since the same sporangial membrane appears in electron microscope pictures taken by other investigators who were not necessarily studying spore formation. Krasilnikov (**1949**), discussing spore formation, states that spores are formed simultaneously. His figure 8 clearly shows that the spores are formed within a membrane comparable to a sporangium, although he does not call the structure a sporangium.

This manner of sporulation assumes added significance when one considers the work of Couch (**1949**) on *Actinoplanes* and *Streptosporangium.* This interesting group of organisms belonging to the Actinomycetales produces zoosporangia and zoospores in liquid culture. When grown on media used for growth of *Streptomyces,* the colonies resemble those of other actinomycetes. He reported that one species formed sporangia which released nonmotile spores. The aerial hyphae resembled that of *Streptomyces,* thus suggesting a connecting link between the zoosporangial forms and *Streptomyces.* Karling (**1954**) also reported a similar type organism which produced sporangia and motile zoospores. He did not name it, but indicated that it belonged in the Actinoplanaceae. Rothwell (**1957**) studied Karling's organism and found the motile spores to have a single polar flagellum. The flagella are unlike those of certain lower Phycomycetes. Interestingly, the sporangium in this organism has its spores aligned in straight rows. It is not difficult to imagine a reduction of the rows to a single linear series of spores within a sporangial wall. Couch (**1955**) formally recognizes a distinct family, Actinoplanaceae, which has true mycelium and whose spores all form in

sporangia. Representative species of the genus *Actinoplanes* contain flagellated motile spores, whereas those of *Streptosporangium*, have nonmotile spores without flagella. The spores of both genera are formed in chains inside sporangia. Couch (**1954**) concluded that, because of their staining properties, the flagella in *Actinoplanes* more closely resemble those of bacteria than those of fungi belonging to the chytrids. However, he points out the striking resemblance of the mycelium and sporangia to that found in the chytrids and concludes that these organisms may represent a connecting link between the bacteria and the lower fungi.

Motility in actinomycetes is not restricted to this family, since Orskov (**1938**) and others have reported motility in proactinomycetes. Jensen (**1953**) examined one of Orskov's strains, probably *Nocardia citrea* (Krassil.) Waks. & Henrici, and confirmed spore motility. Initially, mycelium was produced by this strain but after a few hours some of the surface hyphae divided into rod-shaped cells which became motile when placed in water. The number of flagella varied from one to four per cell. In the genus *Dermatophilus* spores are formed in a peculiar fashion. The club-shaped cells divide both transversely and longitudinally in a manner resembling the septations of *Alternaria* spores. Each cell then produces a single motile zoospore with spiral flagella that vary in number. The manner in which the spore is released is not clear, although Thompson and Bisset (**1957**) believe that the filament simply breaks up and that each segment becomes a motile coccus that forms flagella on its outer wall. On the other hand, Austwick suggests that the divided filaments act as multilocular sporangia with each segment releasing a single spore through a pore or by rupture of the sporangial wall. The colonies of the three species placed in this genus are mycelial and restricted; they appear as typical actinomycete colonies. The production of septa in two directions and the formation of motile spores prompted Austwick (**1958**) to propose Dermatophilaceae as the fifth family in the actinomycetes. Cultures of these organisms are available for further study. As in the Phycomycetes, it is not difficult to imagine that evolution progressed to the point where a linear chain of spores became arranged in a sporangium. The next logical step is found in the genus *Waksmania*, where the spore number in sporangia has been reduced to two. In *Micromonospora* the sporangia are further reduced to contain but a single spore. *Dermatophilus*, on the other hand, stands down the evolutionary scale retaining motile spores found in uniloculate sporangia. It probably did not evolve further since its species are pathogens.

In *Nocardia*, spores are apparently still delimited like those found in *Streptomyces*, but the sporophore is reduced in complexity. However, the increased ability to produce oidia or fragmentation cells serves to enhance reproduction in *Nocardia* species. Some Nocardias are acid-fast, that is, when the cells are stained with fuchsin in phenol the cells retain their stain after washing with acid alcohol. Others are not. From *Nocardia* it is not difficult to image the situation found in species of *Mycobacterium*. Sporangial spore production has been lost in *Mycobacterium* in favor of fragmentation. Cells of this genus are acid-fast due to their unusually high lipid content. Of the 14 strictly aerobic species recognized, 6 are saprophytic and 8 are parasitic. Two of the parasitic species have become so specialized that they never have been grown on nonliving culture media. Such strict parasites could not have evolved except by the evolution of their hosts. It is hard to believe that such specialized microorganisms could have evolved towards free-living forms. Unlike true bacteria, mycobacteria possess cell walls very rich in fats and waxes. Another resemblance between *Mycobacterium* and *Nocardia*, which has been exploited in oil discovery work, is their ability to attack relatively inert substances. Both genera contain species that are able to utilize paraffins, hydrocarbons, benzene and aromatic amines. The remaining pathogens in the actinomycetes are species of *Dermatophilus* and *Actinomyces*. Although similar to *Nocardia*, *Actinomyces* has representatives that are anaerobic and are always associated with animals. The nonacid-fast cells of *Micrococcus* are similar to *Mycobacterium* and must have evolved from nonacid-fast *Nocardia*. *Micrococcus* cells germinate with germ tubes, thus showing further relationships to *Nocardia*. In addition to the above genera, several genera showing parallel relationships have been recognized on the basis of their ability to grow at high temperatures. For example, the genus *Thermoactinomyces* Tsiklinsky duplicates *Micromonospora* except that it contains species which grow at temperatures of 50–65° C. The proposed phylogenetic scheme for this group is shown in FIG. 1.

Thirumalachar (**1955**) demonstrated that certain *Streptomyces* regularly produce sclerotia in culture. The genus *Chainia* was erected to encompass these forms. The sclerotia may reach 75 μ in diameter and are completely similar to comparable structures encountered in the fungi. Gattani (**1957**) also reported the presence of sclerotia in certain strains of *S. griseus* but concluded that there were insufficient grounds for recognizing the genus *Chainia* on the basis of sclerotial production.

Some fungi possess a parasexual means of reproduction (a process which leads to genetic recombinations aside from sexual reproduction).

Parasexuality was found in the actinomycetes by Sermonti and Spada-Sermonti (**1955**). Bradley and Lederberg (**1956**) confirmed that heterokaryosis occurs in *Streptomyces,* and further found that each spore from a prototrophic colony had the characteristics of one of the parents only, while mycelial fragments often were like the prototrophic colony. This

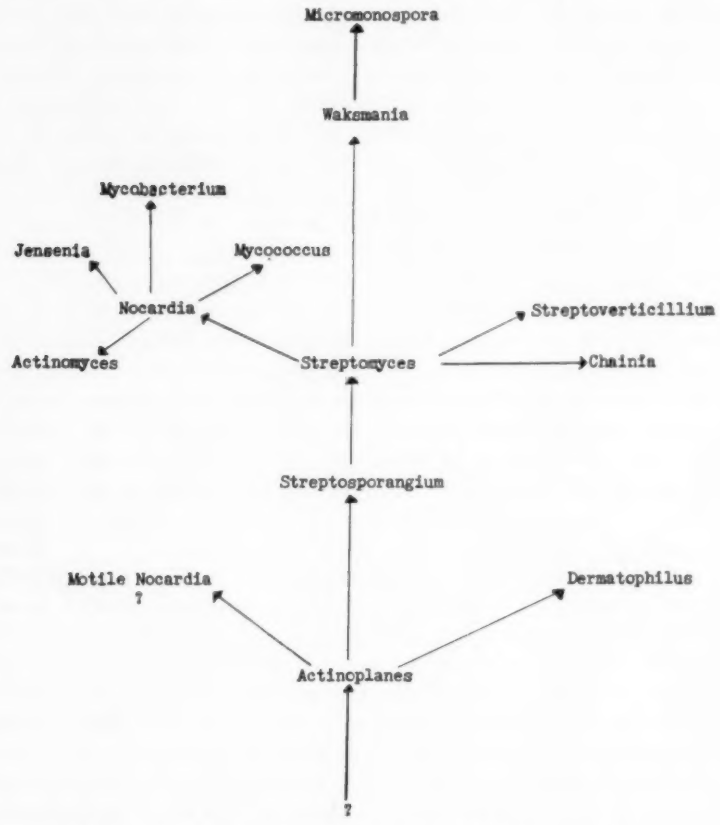

FIG. 1. Possible direction of evolution in the Actinomycetales.

work is additional evidence that spores of *Streptomyces* are uninucleate. Carvajal (**1946**) reported the anastomosis of mycelium of *Streptomyces* but did not illustrate his evidence. Anastomosis of hyphae occurred in the two strains of *Streptomyces* studied by Gregory (**1956**) and he provided excellent photographic evidence.

PHYSIOLOGICAL EVIDENCE

In any study of the relationships of the actinomycetes to other microorganisms a considerable amount of evidence of a nonmorphological nature must be weighed. One of the best arguments in favor of a closer relationship to the bacteria is the susceptibility of Actinomycetales to infection by phages. Although bacteria are susceptible to phages, no confirmed instances of phage-attacking fungi are known. On the other hand, phages attacking actinomycetes have been demonstrated repeatedly. For example, Carvajal (**1953**) gave one of the most complete accounts of the action of phages on industrial-scale *Streptomyces* fermentations. According to him, phage in actinomycetes was first found by Wiebols and Wieringa in 1936. Bradley and Anderson (**1958**) showed that a single actinophage which attacked various species of *Streptomyces* also lysed cells of *Nocardia*. This is further evidence that the distinction between the genus *Nocardia* and *Streptomyces* is not real.

Chemical composition of the cell wall has received much consideration as a means for determining the relationships between bacteria and fungi. Fungal cell walls contain a high amount of chitin, whereas those of bacteria do not. Thimann (**1955**, p. 102) pointed out that chitin occurs in some bacterial cell walls. Certain fungi, including *Saccharomyces* and several other yeasts and *Ustilago violacea* sporidia, do not have either chitin or cellulose, according to Cochrane (**1958**). Avery and Blank (**1954**) could not detect chitin or cellulose in the cell walls of actinomycetes. Their investigations included *Actinomyces, Streptomyces, Nocardia* and *Micromonospora*. Bishop and Blank (**1958**) found a polysaccharide containing D-arabinose and D-galactose in *Nocardia asteroides* and the same compounds have been reported in *Mycobacterium*. This evidence points toward a relationship of *Nocardia* and *Mycobacterium*. Cummins and Harris (**1958**) studied the cell-wall composition of 51 strains of *Actinomyces, Nocardia, Streptomyces, Micromonospora, Mycobacterium, Propionibacterium* and 7 strains of true fungi (*Penicillium, Aspergillus, Rhizopus, Mucor, Microsporum, Trichophyton* and *Epidermophyton*). Whereas the cell walls of actinomycetes consisted of sugars, amino sugars and amino acids, those of the fungi were composed entirely of carbohydrates. As a result of their information on cell-wall composition, they suggested that propionibacteria are probably related to *Streptomyces* and that the recognition of Actinomycetales as a separate order from the Eubacteriales is unnecessary. Sohler, et al. (**1958**) found arabinose, galactose, glutamic acid, diaminopimelic acid and alanine as the principal materials in the cell walls of *Streptomyces, Nocardia* and *Micromonospora*. Several reports occur in the literature

indicating that lysozyme may be used to remove the cell walls of *Streptomyces* as well as those of bacteria. Although chitin appears to be absent from actinomycete cell walls, this is also true of most Oomycetes.

Diaminopimelic acid exists as a compound with three stereoisomers and they are confined in their distribution to bacteria, including *Corynebacterium* and *Mycobacterium*. Hoare and Work (**1957**) studied its distribution among several genera of the Eubacteriales and the Actinomycetales. The following results are summarized from their work:

Microorganism	No. of strains	Diaminopimelic acid isomer		
		Meso	LL	DD
Actinomycetales				
Mycobacteria	6	+++	− −	− −
Nocardia	7	+++	− to trace	− −
Actinomyces	1	− −	+++	− −
Streptomyces	8	− to +	++	− −
Micromonospora	3	++	++	+ −
Eubacteriales				
Propionibacterium	1	− −	+++	− −
Bacillus	3	++	− to +	− −
Clostridium	9	− to ++	− to ++	− −

These data suggest that *Nocardia* and *Mycobacterium* are closely related and the occurrence of the acid in actinomycetes shows a definite relationship to the true bacteria. To date, the only other naturally occurring source of diaminopimelic acid is in the algal genus *Chlorella*.

In an evaluation of the relationships of actinomycetes to other microorganisms, some consideration must be given to fermentation products produced. Of necessity, such a consideration must be based on unusual products encountered in a number of species in the actinomycetes but seldom found elsewhere in other microorganisms. Compounds and metabolic patterns encountered commonly in all organisms would be of no significance. Vitamin B_{12}, an example of a chemical compound in the former class, was first isolated from species of *Streptomyces* and as far as I am aware has not been encountered in fungi except for one report by Tanner (**1960**). Later it was found that species of the bacterial genus *Propionibacterium* are better sources of vitamin B_{12} than *Streptomyces*. The propionic bacteria are nonmotile, Gram-positive, true bacteria which have irregular shaped cells that tend toward bending and terminal swelling. They produce propionic acid. An interesting development was the discovery by Hungate (**1946**) of a species of *Micromonospora* which formed propionic acid. This species was isolated from the alimentary

tract of a termite and unlike most species, grows under anaerobic conditions. Thus, two additional pieces of evidence point toward a relationship between propionic bacteria and actinomycetes. Stanier and van Niel (**1941**) proposed that evolution progressed from the streptococci through the propionic bacteria, corynebacteria, mycobacteria to *Nocardia* (*Proactinomyces*) and thence to *Streptomyces* (*Actinomyces*).

Gram-positive bacteria are generally inhibited by penicillin, which has little or no effect on fungi. Actinomycetes are inhibited by this antibiotic. In general, this appears to be true with other antibiotics, *i.e.*, if they inhibit bacteria they also will inhibit actinomycetes.

Rehm (**1958, 1959**) recently announced some startling and significant discoveries in the biology of *Streptomyces*. In a series of five papers he demonstrated and beautifully illustrated how *Streptomyces* (*S. albus* and *S. griseus*) actively parasitize the mycelium of some fungi both in culture and in unsterile soil. Actinomycete mycelium grew around and about the mycelium of *Aspergillus niger* v. Tiegh. and aggressively attacked it. Earlier observations had shown that some plant pathogenic soil fungi could be controlled by increasing the organic material in infested soil. After this treatment the actinomycete population greatly increased. Control of the plant pathogens may have resulted from the parasitic action of the enhanced *Streptomyces* population. I have observed on plates of hay agar inoculated with soil and incubated 2 or 3 weeks that strands of fungus hyphae extend aerially from one soil particle to another. The hyphae were covered with a down of fine mycelium; this mycelium is actually chains of spores of *Streptomyces*, with the substrate mycelium on and in the host. The parasitizing strains were *S. griseus* just as Rehm reported. Certainly this type of parasitization would appear to be typical of fungi which attack the mycelium of other fungi.

Doubtless, many other morphological and physiological characters might be considered. The uniformly small diameter of the mycelium in the Actinomycetales is stated to indicate bacterial relationships. Some have claimed the sensitivity of representatives of this order to acids would also indicate bacterial affinities. However, certain fungi are known to be extremely sensitive to acid, and still some *Streptomyces* will grow under acid conditions. Extreme care must be taken, especially on physiological grounds, in drawing conclusions as to natural relationships. Some types of antibiotics, particularly the highly unsaturated acetylenic ones, are found in both *Streptomyces* and Basidiomycetes. Still, no one would conclude that the two groups are related. Another example of this type is found in the quinone pigments found occurring naturally in both actinomycetes and fungi.

SUMMARY

The evolutionary scheme herein proposed involves a series of organisms showing morphological and physiological relationships beginning with a sporangial ancestor with motile spores evolving to reduced spore production and nonmotile spores. This scheme seems best to fit the facts as they exist today. Although related both to fungi and to bacteria, especially the propionic bacteria, the actinomycetes would appear to be a separate phylogenetic line. Whether this theory is acceptable or not will await many more comparative studies of morphology, physiology and chemistry.

From a practical standpoint we are certain, however, that actinomycetes must be isolated, grown and studied more as if they were fungi than bacteria. This is because: (1) Spores and spore germination are like those of fungi. (2) Vegetative growth in surface and submerged culture is fungus-like. (3) Mycological characteristics used in identification seem most fundamental. (4) Methods required for maintaining vigorous and stable cultures are like those used for fungi. (5) The colony and its mode of growth are more fungus-like than bacterial.

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JAMESDICKSONIA, A NEW GENUS OF THE USTILAGINALES

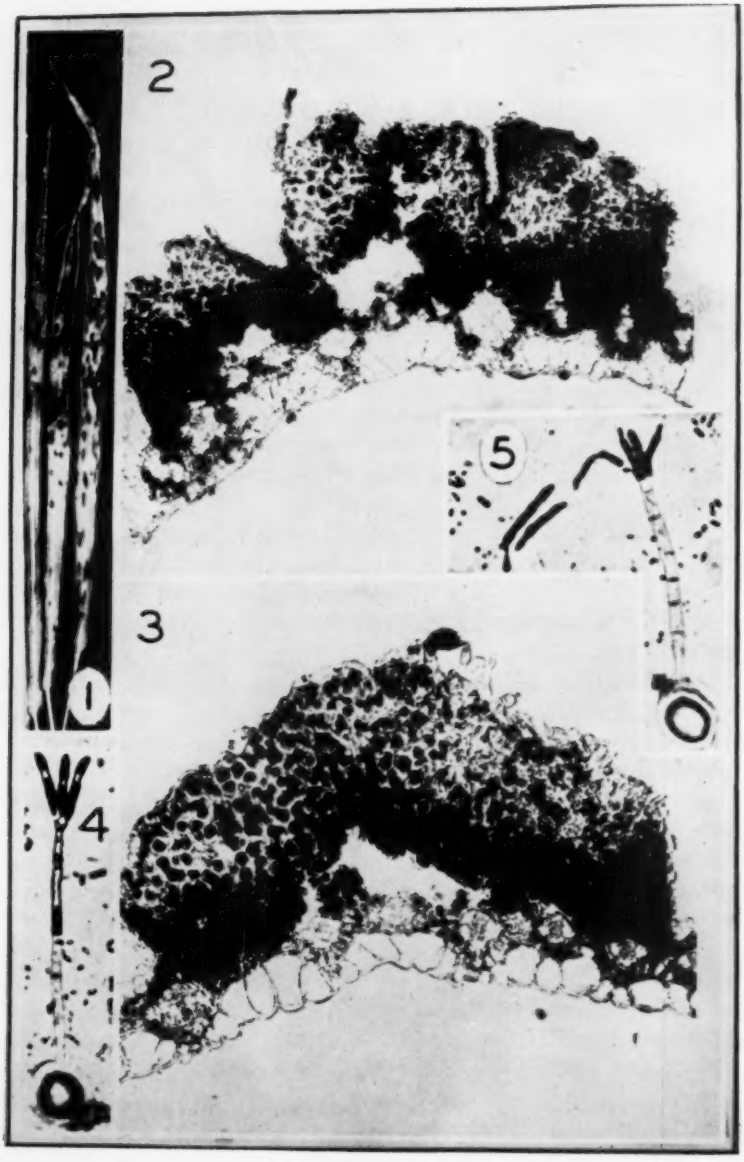
M. J. Thirumalachar, M. S. Pavgi, and M. M. Payak

(with 5 figures)

In studies of Indian smuts, a leaf smut on *Dichanthium annulatum* (Forsk.) Stapf was collected in several places. Examination revealed it to be *Tolyposporella obesa* (Syd.) Clint. & Zundel. The smut was first collected by Butler in India and described by Sydow (**2**) as *Entyloma obesum* on the basis of resemblance to other *Entyloma* species on grasses. Clinton and Zundel (**6**) transferred it to *Tolyposporella* on account of the production of tar spot-like sori on the leaves and agglutinated spore masses appearing as spore balls. Neither Sydow nor Clinton and Zundel have described the morphology of the sorus or the germination of teliospores. Fresh material was available, and studies of spore development and spore germination indicated that the smut belonged to an undescribed genus. An account of these studies is presented here.

Dichanthium annulatum is a good forage grass in India and remains green even in late summer. It is valuable in pastures, and institutions in India are working on obtaining improved clones. The smut is distributed in several parts of North and South India (**1**, **7**). Infection appears as tar-like spots (Fig. 1) which are slightly raised and up to 5 mm in diameter. When fresh, the hypophyllous sori appear as black hemispherical encrustations closely appressed to the leaf. In old herbarium material, the raised sori appear flattened but the characteristic features remain.

Examination of the type of *Entyloma obesum*, collected near Nagpur, India, confirmed that the collections made on *Dichanthium annulatum* from different areas in India were the same. Sections through the sori at various stages of maturity showed quite unusual features. In a young sorus, strands of hyphae aggregate and develop into stromata beneath the epidermis at two or three places. The hyphae emerge at each site and coalesce with the neighboring groups forming a small umbonate to spherical structure (Fig. 2). Following a rapid growth of this hyphal mass and the differentiation of spores from the outer layer



FIGS. 1–5.

of cells, a flabelliform crust soon develops. The outer layers of hyphae gelatinize and successively differentiate spores in basipetal succession, so that the spore crust appears similar to that observed in the rust genus *Dasturella*. In a mature sorus, strands of hyphae emerging from the stomata at several points appear as foot-like connections buried in the host and supporting the flabelliform crust above the epidermis (FIG. 3). Only the upper portion is sporiferous, while the remaining tissues gelatinize and collapse following the germination of spores and subsequent disintegration of the sori.

The spores are never borne in spore balls, but in scraped mounts spore agglutinations become detached from the flabelliform crust and appear in groups. Mature spores are dark reddish brown to olive-brown, subglobose to ellipsoidal, smooth, and 14 to 30 μ in diameter. The thick hyaline epispore swells in water up to 15 μ and shows a lamellated structure. The spores were germinated and stained by the method of Thirumalachar and Narasimhan (**5**). The mode of germination is characteristic of the Tilletiaceae, with a promycelium bearing a whorl of 4 to 8 sporidia (FIG. 4), which do not conjugate but produce secondary sporidia (FIG. 5). These, when detached from the primary sporidia, fuse in pairs and develop the infection hyphae.

The characters of the sorus are not duplicated in any named genus of the smuts. The general appearance of the sorus resembles that in *Kweilingia*, described by Teng (**3**) as a genus of the Uredinales on leaf sheaths of bamboo in China. Thirumalachar and Narasimhan (**4**), who studied the type of the genus, *K. bambusae* Teng, showed that it was not a rust, since strands of hyphae emerged out of the stomata and coalesced above the epidermis to form flabelliform crusts. Spores are differentiated from the upper layers of the crust, leaving the lower portion as a plectenchymatic mass. The germination, however, is with a septate promycelium bearing lateral and terminal sporidia as in *Phragmobasidium*. Thirumalachar considered that *Kweilingia* might be placed under the Auriculariales. However, with another closely related smut belonging to the Tilletiaceae, it is now proposed to include *Kweilingia*

FIGS. 1–5. *Jamesdicksonia obesa*. FIG. 1. Tar spot-like crustaceous sori on the leaves of *Dichanthium annulatum* (× about half nat. size). FIG. 2. Cross section through a young sorus showing hyphal strands in the host tissue, coalescent umbonate sori and basipetal differentiation of spores in the sorus. FIG. 3. Cross section through a mature sorus showing mass of mature spores forming a hypophyllous encrustation and foot-like structures in the leaf tissue. FIG. 4. Germinated teliospore showing hyaline swollen epispore and promycelium bearing a whorl of sporidia. FIG. 5. Sporidia budding *in situ* and after detachment.

as a genus of the Ustilaginaceae. The smut on *Dichanthium annulatum* represents an undescribed genus of the Tilletiaceae. The name *Jamesdicksonia* is proposed in honor of Dr. James G. Dickson, University of Wisconsin, eminent student of fungi and inspiring professor.

JAMESDICKSONIA gen. nov.

Sori in crustis erumpentibus, conicis obconicis usque flabelliformibus dispositi, ex hyphis compactis emergentibus, sursum crescentibus, coalescentibus et demum crustas umbonatas supra epidermidem efformantibus compositi; teliosporae deinceps basipetaliter e cellulis hyphalibus gelatinosis e superficie crustae natae; sporae inter se adhaerentes nec in maturitate disjungentes, unicellulares, subhyalinae, e promycelio verticillum sporidiorum 3–8 ferente sicut in Tilletiaceis germinantes.

Sori occurring as crusts, erumpent, conical, obconical to flabelliform, formed by mass of hyphal tissue emerging and growing upwards and coalescing with neighboring groups to develop umbonate crusts above the epidermis. Teliospores formed in basipetal succession followed by gelatinization of the outer hyphal cells of the crust; spores adhering en masse and not separating out at maturity; one-celled, subhyaline and germinating by a promycelium bearing a whorl of 3 to 8 sporidia as in Tilletiaceae.

Type species: *Jamesdicksonia obesa* (Syd.) comb. nov.

Jamesdicksonia obesa (Syd.) comb. nov.

Entyloma obesum Syd., Ann. Mycol. **9**: 145. 1911.
Tolyposporella obesa (Syd.) Clint. & Zundel, Mycologia **22**: 157. 1930.

Sori in the leaves, tar spot-like, hypophyllous, light reddish brown, elongated up to 4 mm, raised and erumpent; strands of hyphae emerging and growing upwards, forming conical, obconical crusts. Teliospores adhering en masse, dark reddish brown to olive-brown, subglobose to ellipsoidal, smooth 14 to 30 μ in diameter; epispore swelling in water often up to 15 μ in thickness with characteristic striations. Spores germinate as in Tilletiaceae with a promycelium bearing a whorl of 4 to 6 sporidia which successively bud off secondary sporidia.

In living leaves of *Dichanthium annulatum* (Forsk.) Stapf at Nagpur (*Pandit*), Dehra Dun (*Butler*), Mussoorie (*Pavgi*), Majhgawan, M. P. (*Pavgi*), Dohad (*Butler*), Poona (*Thirumalachar*).

We are grateful to Miss Edith K. Cash for translating the diagnosis

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THE EFFECT OF AMMONIA ON GROWTH AND REPRODUCTION OF PILOBOLUS KLEINII

ROBERT M. PAGE

(WITH 6 FIGURES)

Pilobolus kleinii van Tieg., a coprophilous phycomycete of the order Mucorales, can be grown on a synthetic medium which contains asparagine, sodium acetate, hemin, thiamine, and salts (Page, **1952**). When the fungus is grown on this medium in an atmosphere containing certain concentrations of ammonia, or when the medium is supplemented with the proper concentrations of ammonium salts, asexual reproduction is greatly stimulated (Page, **1959**). The purpose of this paper is to report the effect of ammonium ion on sexual reproduction, asexual reproduction, and vegetative growth and to consider the role of ammonia in the nutrition of *P. kleinii*.

MATERIALS AND METHODS

The strain of *Pilobolus kleinii* employed for trials involving vegetative growth and asexual reproduction was the same as that used in previous work (Page, **1959**). It was isolated from cow dung collected near Two Rock, Sonoma County, California, July 18, 1956 and was arbitrarily designated as (+). For experiments on sexual reproduction, this isolate was mated with a (−) strain which was isolated from cow dung collected near Valley Ford, Sonoma County, California, on the same day. These strains have been deposited with the American Type Culture Collection where they have been assigned the numbers ATCC 13701 and 13700, respectively.

The basal medium employed for this study contained (per liter): L-asparagine, 7.55 g; sodium acetate (anhydrous), 6.03 g; hemin, 10 mg (dissolved in 37.5 ml N/10 NaOH); thiamine·HCl, 10 mg; KH_2PO_4, 1.0 g; K_2HPO_4, 1.2 g; $MgSO_4 \cdot 7H_2O$, 1.0 g; NaCl, 0.1 g; $CaCl_2$, 0.1 g; and 1.0 ml of a micronutrient solution (Ryan et al., **1943**). For tests on sexual or asexual reproduction, the medium was solidified with 2 per cent agar. In some cases, as will be indicated later, asparagine was omitted and the concentration of sodium acetate varied.

For determinations of vegetative growth, *P. kleinii* was grown in 20 ml of liquid medium contained in 125-ml Erlenmeyer flasks. The medium in each flask was inoculated with a block of agar containing hyphal tips cut from the margin of an actively-growing mycelium. The cultures were incubated at 25° C in the dark for eight days. The mycelia were harvested by collecting them on filter paper in a Buchner funnel. The agar blocks were dissected away, and the mycelia were rolled into balls which were dried at 60° C and weighed.

The intensity of asexual reproduction was estimated from cultures grown in agar media in 60 mm Petri dishes. The medium in each dish was inoculated with a block of agar containing hyphal tips, and the cultures were incubated in the dark for three days. They were then exposed to light (150 ft-c) for 100 seconds to induce the formation of trophocysts (Page, **1956**) and were returned to darkness for 24 hours. Trophocysts were counted with the aid of a low-power magnifying lens. When trophocysts were numerous, their number was estimated by counting trophocysts in two sectors each of which occupied $\frac{1}{8}$ of the area of the bottom of the dish and multiplying the sum by four.

The number of zygospores formed on agar media in 100 mm Petri dishes served as a measure of sexual reproduction. The cultures, which were inoculated by placing agar blocks containing hyphal tips of the two compatible strains two cm apart on the surface of the medium, were incubated in the dark for four days at 25° C. The zygospores were counted with the aid of a dissecting microscope.

All treatments were replicated four times.

RESULTS

The effect of ammonia on growth and reproduction of *P. kleinii* was tested by adding ammonium sulfate to the basal medium containing asparagine to give a range of concentrations of ammonium ion. The results of typical trials are shown in FIG. 1. These results confirmed those previously reported (Page, **1959**) with respect to asexual reproduction in that they showed that ammonium ion produced a marked stimulation of trophocyst formation at certain concentrations. Low concentrations of ammonium ion over a considerable range had only a slight effect on the number of trophocysts formed, but concentrations near 0.01 M NH_4^+ were strongly stimulatory. Higher concentrations of ammonium ion produced less stimulation, and still higher concentrations suppressed trophocyst formation entirely.

Addition of ammonia to the asparagine medium also affected sexual reproduction, and, as was the case with asexual reproduction, there was

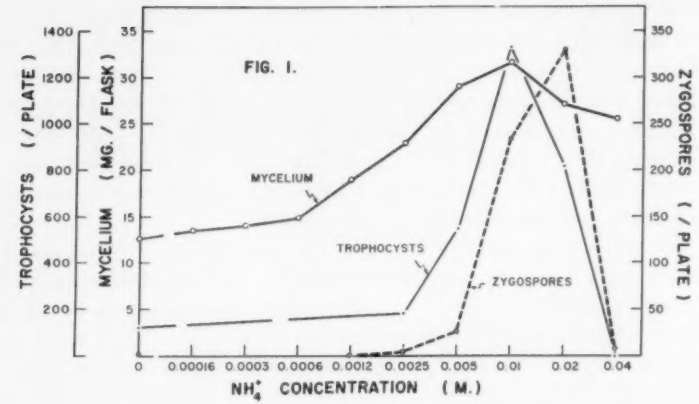
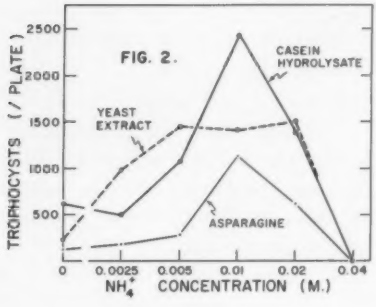
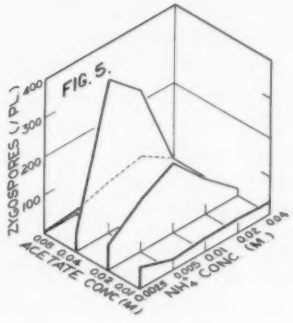
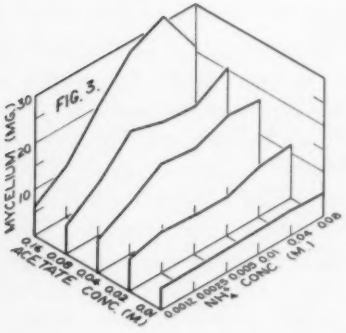
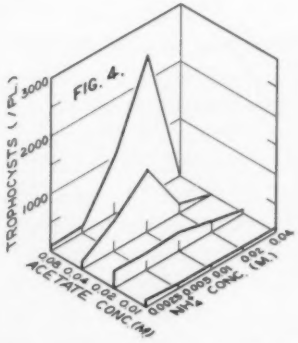


FIG. 1.
TROPHOCYSTS (/ PLATE)
MYCELIUM (MG. / FLASK)
ZYGOSPORES (/ PLATE)
MYCELIUM
TROPHOCYSTS
ZYGOSPORES
0 0.00016 0.0003 0.0006 0.0012 0.0025 0.005 0.01 0.02 0.04
NH4+ CONCENTRATION (M.)









FIGS. 1–5. FIG. 1. Effect on growth, asexual reproduction, and sexual reproduction of *Pilobolus kleinii* of addition of various concentrations of ammonium ion to a basal medium containing asparagine and sodium acetate. FIG. 2. Effect on asexual reproduction of *P. kleinii* of addition of various concentrations of ammonium

a pronounced effect of concentration. Low concentrations of ammonium ion had little effect on the number of zygospores formed; certain concentrations greatly stimulated zygospore formation, and still higher concentrations inhibited the production of zygospores.

Vegetative growth was also influenced by the addition of ammonium ion to the basal medium containing asparagine, but the effect of concentration was somewhat different than that on reproduction. Even relatively low concentrations of ammonium ion brought about detectable increases in the weight of mycelium, and increasing the concentration increased the amount of growth until an optimal concentration was reached. Concentrations of ammonium ion which exceeded this optimal range were less stimulatory, and still higher concentrations were toxic.

Thus, ammonia stimulated growth and sexual reproduction as well as asexual reproduction, but the three processes were affected differently by various concentrations of ammonium ion. Relatively low concentrations of ammonium ion increased the amount of vegetative growth but had little effect on reproduction. Again, growth was vigorous at concentrations which exceeded the optima for trophocyst and zygospore formation. The effect of concentration was most striking in the case of reproduction, because trophocyst and zygospore formation were stimulated greatly—but only over a relatively narrow range of ammonium ion concentrations.

Since ammonia proved stimulatory to growth as well as to reproduction, it was of interest to attempt to elucidate the role of ammonia in the nutrition of *P. kleinii*. The following three possibilities were considered: that ammonia exerts its effect by altering the pH of the medium; that ammonia circumvents a block in a pathway leading to the synthesis of some particular type of cellular material, and that ammonia serves as a general nitrogen source. For most of these trials, asexual reproduction was studied, because the effect of ammonia on trophocyst formation is clear-cut and easily investigated.

Effect of pH.—In order to test the effect of addition of ammonia on the pH of the medium and on trophocyst formation, various concentrations of ammonium tartrate were added to the basal medium. The in-

ion to basal media containing sodium acetate and asparagine, casein hydrolysate, or yeast extract. FIG. 3. Effect of various concentrations of ammonium ion and acetate on vegetative growth of *P. kleinii*. Isometric projection. FIG. 4. Effect of various concentrations of ammonium ion and acetate on asexual reproduction of *P. kleinii*. Isometric projection. FIG. 5. Effect of various concentrations of ammonium ion and acetate on sexual reproduction of *P. kleinii*. Isometric projection.

itial pH of the sterile medium was determined before the agar gelled, and the final pH of the medium was measured at the end of the trial by pooling the media in the four replicate dishes, macerating the agar in water, and determining the pH of the aqueous extract.

The results (TABLE I) indicated that there was no correlation between either the initial or the final pH of the medium and the number of trophocysts formed by *P. kleinii*. Over the range of concentrations employed, the addition of ammonium tartrate to the basal medium altered the initial pH but little. In all cases the medium became alkaline after the fungus had grown on it—presumably as a result of the utilization of acetate by the fungus. The lack of correlation between pH and the intensity of asexual reproduction was shown most clearly by the results with 0.005 and 0.015 M ammonium ion. Both the initial and the final

TABLE I

THE EFFECT OF VARIOUS CONCENTRATIONS OF AMMONIUM ION ON TROPHOCYST FORMATION AND ON THE PH OF SYNTHETIC MEDIUM CONTAINING ASPARAGINE

Ammonium ion concentration (M)	pH		Trophocysts (per plate)
	Initial	Final	
0	6.95	8.4	109
0.0005	6.95	8.4	91
0.0015	7.0	8.7	90
0.005	7.0	9.0	86
0.015	7.0	9.0	766
0.05	6.9	8.6	0

pH were the same at these concentrations, but the number of trophocysts differed greatly.

Further evidence that ammonia does not stimulate trophocyst formation by altering the pH of the medium is the fact that substitution of sodium or potassium salts for ammonium salts changed the pH of the medium, but only ammonium salts stimulated trophocyst formation (Page, 1959).

Substitutes for Ammonia.—It was conceivable that ammonia was exerting its stimulatory effect by circumventing a bottleneck in transamination or in the synthesis of some class of cellular constituents. Very crudely, cellular constituents might be expected to fall into three classes: proteins, cell wall constituents, or nucleic acid components. To test the possibility that the synthesis of one of these classes might be limiting asexual reproduction, casein hydrolysate, yeast extract, glu-

cosamine, and cytidine monophosphate were added to the basal medium in a range of concentrations. None of these additions stimulated trophocyst formation. Casein hydrolysate produced little increase in the number of trophocysts when it was added in concentrations equivalent to ammonium ion concentrations which produced marked increases. Even when casein hydrolysate (1%) or yeast extract (1%) were substituted for asparagine, addition of ammonium ion stimulated trophocyst formation (FIG. 2), but the concentrations required were lower. Similarly, cytidine monophosphate (tested by adding various concentrations to 1.0 ml portions of basal medium in small tubes) was without effect; whereas, ammonium ion was stimulatory when tested by the same technique. The only materials which were stimulatory were either ammonium compounds or those, such as urea, which yield ammonia readily.

Carbon-nitrogen Balance.—Since it appeared that ammonia might be serving as a general nitrogen source, it seemed possible that some of the stimulation of growth and reproduction observed when ammonia was added to the basal medium containing asparagine might be attributed to changes in the carbon-nitrogen balance. In order to test this possibility, asparagine was omitted from the basal medium, and the concentrations of ammonium ion (supplied as ammonium sulfate) and acetate were varied within wide limits.

The results of representative individual trials of the effects of ammonium-acetate balance on growth, asexual reproduction, and sexual reproduction are shown in FIGS. 3, 4, and 5. In addition, the data from all trials have been averaged and combined in FIG. 6 in order to facilitate interpretation of the results.

Vegetative growth occurred over a wide range of ammonium ion and acetate concentrations (FIG. 6). 0.16 M ammonium ion and 0.64 M acetate were toxic, but some growth, at least, occurred at all other concentrations tested. Interactions between the carbon and nitrogen sources are most clearly shown in FIG. 3. At low levels of acetate, increase in the concentration of ammonium ion produced only slight changes in the weight of mycelium, and similarly, when ammonium ion was limiting, changes in the acetate concentration had little effect on growth. When one component was not limiting, increases in the other up to an optimal concentration increased the amount of growth. The optimal concentration of one component depended, in some instances, on the concentration of the other component. Growth was most luxuriant in media which contained 0.02 M ammonium ion and 0.16 M acetate.

Asexual reproduction occurred over a slightly narrower range of ammonium ion and acetate concentrations than did vegetative growth,

but the optimal concentrations were approximately the same (FIG. 6). No trophocysts were formed in media which contained 0.08 M ammonium ion, but some trophocysts, at least, were formed at all other concentrations which supported growth. As was the case with growth, changes in the concentration of one constituent produced little change in

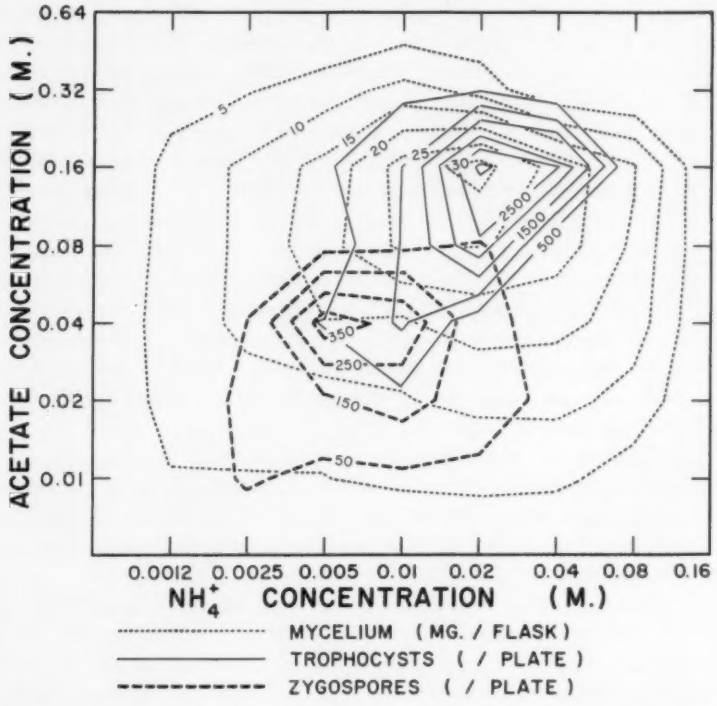


FIG. 6. Diagram showing the effects of various concentrations of ammonium ion and acetate on growth, asexual reproduction, and sexual reproduction of *Pilobolus kleinii*. Contour lines connect points of equal response interpolated from the averaged data of several experiments.

the number of trophocysts formed when the other component was limiting, but the effect of interactions at other concentrations was more spectacular (FIG. 4). At moderately high concentrations of acetate (particularly 0.04 and 0.08 M), increasing the concentration of ammonium ion had little effect on the numbers of trophocysts formed until a critical level was reached, but further increase in the ammonium ion

concentration produced striking increases in the numbers of trophocysts formed. The shape of these curves was so similar to those observed in previous trials with media containing asparagine, that it would seem probable that their shape was the result of the interaction of the concentrations of carbon and nitrogen sources. The acetate concentration in the earlier trials, incidentally, was approximately 0.6 M which would lie in the region where stimulation was great only over a relatively narrow range of concentrations.

Sexual reproduction—like trophocyst formation—was vigorous over a range of ammonium ion and acetate concentrations that was less extensive than the range over which vegetative growth occurred (FIGS. 5, 6). Although the ranges overlapped, it was evident that the optimal concentrations for zygospore formation were lower than those for growth and trophocyst formation. Since the optimal concentrations of ammonium ion (0.005 M) and acetate (0.04 M) were in the same ratio to each other as the optimal concentrations for growth and trophocyst formation, the dilute medium appeared to be more important in favoring sexual reproduction than the carbon-nitrogen ratio.

DISCUSSION

The results of this investigation showed, first, that ammonium ion stimulates growth and sexual reproduction as well as asexual reproduction of *Pilobolus kleinii*. Second—unlike the situation in *Neurospora* described by Ryan and Schneider (**1947**)—the ammonia did not exert its effect by altering the pH of the medium. Third, from the results of limited trials with casein hydrolysate, glucosamine, yeast extract, and cytidine monophosphate, it would appear that ammonia was not circumventing a reaction which limited the rate of synthesis of one class of cellular constituents. Ammonium ion was preferable to asparagine or amino acids as a nitrogen source, either because *P. kleinii* is not capable of deamidating asparagine or deaminating amino acids readily, or because the hyphal membranes are more permeable to ammonium ion than to these compounds.

It was evident from the results of trials in which ammonium ion served as the sole source of nitrogen that carbon-nitrogen balance and the concentration of the medium affect growth and reproduction of *P. kleinii*. This species of *Pilobolus* thus resembles other fungi, such as *Neurospora* (Westergaard and Mitchell, **1947**; Hirsch, **1954**), in that the carbon-nitrogen balance influences reproduction. With respect to concentration of the medium, *P. kleinii* behaves quite differently from

Sporodinia grandis Link in which zygospore formation increased with the concentration of the medium; whereas, sporangia were formed most abundantly on dilute media (Baker, **1931**). The response of *P. kleinii* is more nearly like that of *Phycomyces blakesleeanus* Burgeff which does not require large quantities of dextrose and aspartic acid for the production of an optimum crop of zygospores (Leonian and Lilly, **1940**); however, *Phycomyces* differs from *P. kleinii* in that higher concentrations of nutrients do not appear to inhibit zygospore formation. Moreover, it would appear from the results of Burkholder and McVeigh (**1940**) that vegetative growth of *Phycomyces* is less sensitive than that of *P. kleinii* to changes in the concentration of carbon and nitrogen sources in the medium.

The fact that mycelial growth occurred over a wider range of ammonium ion and acetate concentrations than either sporangium formation or zygospore production agrees with the generalization that the range of conditions for vegetative growth is wider than the range of conditions for reproduction (cf Hawker, **1957**). Perhaps the requirement of a relatively high concentration of nutrients for optimal growth and asexual reproduction could be regarded as an adaptation of *P. kleinii* to its habitat, because although the amount of soluble nutrients in the dung of herbivorous animals must be low, the amount of water is also small, so that the concentration of such solutes as ammonium ion and acetate may be relatively high. It is more difficult to reconcile the requirement of *P. kleinii* for a dilute medium for optimal sexual reproduction with its ecology.

Whatever the ecological significance of the results, they are of practical importance, because they make it possible to design media which favor specific activities of *P. kleinii*. Together with suitable programs of light and mating, these media should facilitate studies on various aspects of sexual and asexual reproduction by making it possible to produce luxuriant vegetative growth, abundant sporangia, or numerous zygospores at will.

SUMMARY

The weight of mycelium and the numbers of trophocysts and zygospores produced by *Pilobolus kleinii* were greatly increased when certain concentrations of ammonium ion were added to a basal medium which contained asparagine, sodium acetate, hemin, thiamine, and salts. The stimulation did not appear to be due to changes in the pH of the medium. From the fact that casein hydrolysate, yeast extract, glucosamine, and cytidine monophosphate did not duplicate the effect of ammonium ion,

it was concluded that ammonium ion was preferable to these substances as a source of nitrogen.

The results of trials in which ammonium ion served as the sole source of nitrogen indicated that the pattern of stimulation was affected by the concentration of carbon source (acetate). The optimal concentrations of acetate (0.16 M) and ammonium ion (0.02 M) were the same for growth and asexual reproduction, but zygospore formation was favored by a more dilute medium (0.04 M acetate and 0.005 M ammonium ion).

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FACTORS AFFECTING THE GROWTH AND PIGMENTATION OF CLADOCHYTRIUM REPLICATUM [1]

SOLOMON GOLDSTEIN

(WITH 1 FIGURE)

Presumptive evidence that *Cladochytrium replicatum* could be cultivated on an artificial medium was first offered by Sparrow (**1931**), who succeeded in growing the chytrid on corn meal agar. However, the relatively slow growth of the fungus as compared with that of the surrounding contaminants prevented him from obtaining a pure culture. Karling (**1935**), in an account of the distribution of this organism in bodies of fresh water in and around New York City, concluded that it was an ubiquitous saprophyte. He cultivated it on several complex media of which soil mannite agar was best since the mannite medium appeared to retard bacterial growth. It remained for Couch (**1939**) to be the first to achieve success in establishing a pure culture of this or any other chytrid and in his classic paper he outlined several techniques for isolating lower Phycomycetes.

The present investigation was begun in order to expand our knowledge of the factors affecting the growth of this common water chytrid and to ascertain its place within recently propounded phylogenetic schemes (Crasemann, **1954**; Cantino, **1955**; Cantino and Turian, **1959**) based on nutritional characteristics.

MATERIALS AND METHODS

The fungus employed in this investigation was *Cladochytrium replicatum* Karling, a member of the Cladochytriaceae. It is an inoperculate,

[1] This paper is a portion of a dissertation submitted to the Horace H. Rackham School of Graduate Studies of the University of Michigan in partial fulfillment of the requirements for the degree of Doctor of Philosophy. I want to thank Professor F. K. Sparrow, Jr. for his help and encouragement throughout the course of this work. I wish also to express my thanks to Professor A. Geoffrey Norman for his helpful suggestions and for placing the facilities of the Plant Nutrition Laboratory at my disposal. I am indebted to Dr. Robert J. Lowry for the photomicrograph and to Dr. Melvin S. Fuller of Brown University for the isolate of *Cladochytrium replicatum*. Grateful acknowledgment is made of the financial support given by the Department of Botany of the University of Michigan. Contribution from the Department of Botany, University of Michigan No. 1127.

polycentric chytrid which bears numerous septate turbinate organs along its rhizoidal thallus (FIG. 1). In nature the fungus develops an orange pigment in portions of its rhizomycelium and in the zoospores.

It was cultivated in a glucose-tryptone-yeast extract broth, Medium A (TABLE I), and fragmented for use as inoculum in a manner described elsewhere (Goldstein, 1961). A test of the reliability of the inoculation techniques revealed that, employing 2 ml of inoculum, the differences observed between the means derived from duplicate experiments could be attributed to random errors in sampling in better than 20% of the

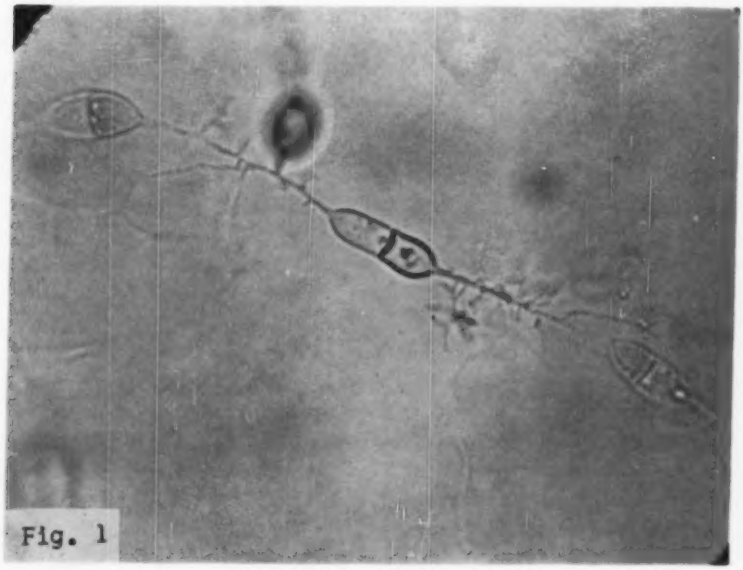

FIG. 1. Rhizomycelium of *Cladochytrium replicatum* grown in stationary, nutrient broth for 16 days. Note the septate turbinate organs. × 1100.

trials. No coefficient of variation exceeded 10%. Unless otherwise stated the components of each nutrient solution were autoclaved together at 15 lb pressure for 15 min since this did not adversely affect the yields of the fungus. After inoculation, the volume in the 125 ml experimental flasks was 40 ml. In general, the chytrid was grown at 21° C in the dark and was harvested after a 16 day incubation period at which time yields were maximal. Dry weight was used as the index of growth and the results are expressed as the arithmetic means derived from three to six replicates per experiment.

The chemicals employed in this study were of reagent grade. The majority of the organic compounds were obtained from the Nutritional Biochemicals Corporation. The sources of the others are as follows: arabinose, soluble starch, and dulcitol—(Pfanstiehl Laboratories); cellophane—(300 Put-0)—Du Pont (Yerkes Research Lab., Film Dept.); fructose—(Mann Research Laboratory); galactose—(Eastman Organic Chemicals Company); gluconic acid—(K & K Laboratory); yeast extract, tryptone, and agar—(Difco Laboratories). Liquid Carbonic Corp. nitrogen (99.9%) was employed. Glassware was scrubbed with Wyandotte Dural H and warm water. This was followed by several rinses in dilute HCl, tap water, and distilled water. The glassware was then permitted to drain dry.

RESULTS

Utilizing Medium A investigations designed to indicate optimal conditions for cultivating *C. replicatum* revealed that it grew best from 15° to 25° and gave meager yields at 5° and 30° C. Harvests were most abundant in media with a pH of between 5.9 and 7.0. Subsequently, therefore, nutrient solutions were adjusted to an initial pH of 6.4 by means of phosphate buffer, the final concentration of which was .02M. Although the tendency of the chytrid to ramify deeply within tubes of nutrient agar suggested that it might be a facultative anaerobe, it did not grow when cultivated under a nitrogen atmosphere from which oxygen was removed by passage through strongly alkaline pyrogallol. An attempt to increase harvests by incubating the fungus in shake culture was unsuccessful. Indeed, it was observed that media containing yeast extract or tryptone supported slightly lower yields in shake as compared with stationary liquid culture.

In all of the aforementioned studies the chytrid did not become colored. Furthermore, it is interesting to note that a marked increase occurred in the production of turbinate organs in Medium A as compared with the number developed in soil cultures.

In the course of investigating the growth factor requirements of *C. replicatum* a vitamin-enriched casein hydrolysate nutrient solution, Medium B (TABLE I), was employed. Its use resulted in more abundant growth of the fungus particularly in shake cultures. In addition, thalli cultivated in this agitated medium grew in the form of star-shaped masses 2 to 6 mm in diameter and developed the orange pigment characteristic of the fungus in nature. In stationary culture, however, the chytrid continued to develop as a white rhizomycelial mat. Light was not found to influence pigmentation and thalli grown in stationary liquid cultures did not become colored in any of the media employed.

Utilizing Medium B enriched with 200 μg of each of the growth factors listed in TABLE II, it was found that *C. replicatum* was dependent upon its environment for thiamin. A quantitative study showed that the incorporation of 50 μg of thiamin per liter of vitamin-deficient Medium B resulted in maximal yields of the fungus.

TABLE I

COMPOSITION OF CULTURE MEDIA

Medium A	
Glucose	5.0 g
Tryptone	4.0 g
Yeast extract	1.0 g
$MgSO_4$	0.5 g
K_2HPO_4	1.0 g
Mineral solution A[a]	5.0 ml
Mineral solution B[b]	1.0 ml
Mineral solution C[c]	1.0 ml
Distilled water	993.0 ml
Medium B	
Glucose	5.0 g
Casein hydrolysate (10% solution, acid hydrolyzed)	15.0 ml
L-Tryptophan	10.0 mg
$MgSO_4$	0.5 g
$K_2HPO_4 \cdot 3H_2O$	1.28 g
KH_2PO_4	1.96 g
Mineral solution A[a]	5.0 ml
Mineral solution B[b]	1.0 ml
Mineral solution C[c]	1.0 ml
Distilled water	943.0 ml

[a] Mineral solution A

H_3PO_3	6.0 mg
$CuSO_4 \cdot 5H_2O$	19.0 mg
H_2MoO_4 (84%)	5.0 mg
$MnSO_4 \cdot H_2O$	50.0 mg
$ZnSO_4 \cdot 7H_2O$	79.0 mg
Distilled water	100.0 ml

[b] Mineral solution B

$FeCl_3 \cdot 6H_2O$	0.2 g
Distilled water	100.0 ml

[c] Mineral solution C

$CaCl_2$	1.0 g
Distilled water	100.0 ml

The ability of the chytrid to assimilate a number of different nitrogen compounds was tested. The nutrient solution employed was Medium B in which one of several nitrogen sources was substituted for casein hydrolysate and tryptophan. In those instances where $(NH_4)_2SO_4$ was used magnesium was added to the mineral solution as the chloride

TABLE II

EFFECT OF VITAMINS UPON THE GROWTH OF CLADOCHYTRIUM REPLICATUM[a]

Vitamins added	Dry weight in mg
None	<1.0
All vitamins listed below	60.8
All but thiamin	1.0
All but nicotinamide	66.0
All but p-amino benzoic acid	68.7
All but ascorbic acid	74.3
All but riboflavin	70.5
All but calcium pantothenate	72.9
All but folic acid	66.7
All but pyridoxine	71.6
All but biotin	74.0
All but inositol	66.0

[a] Cultivated in shake culture at 21° C for 16 days in a vitamin-free casein hydrolysate medium.

rather than as the sulfate. Individual nitrogen sources were added at a concentration of 100 mg of nitrogen per liter of medium. This was doubled, however, when a racemic mixture of an amino acid was employed. The results (TABLE III) revealed that *C. replicatum* could utilize nitrogen in the form of inorganic salts.

Modified Medium B, in which $(NH_4)_2SO_4$ served as the source of nitrogen, was used in surveying the assimilability of various carbon sources. Because the addition of 2 g of carbon as glucose per liter of medium resulted in optimal yields of the fungus, this concentration was employed in testing other organic compounds. It cannot be asserted

TABLE III

THE EFFECT OF VARIOUS NITROGEN SOURCES UPON THE GROWTH OF CLADOCHYTRIUM REPLICATUM[a]

Nitrogen sources	Dry weight in mg
None	2.4
KNO_3	11.1
$(NH_4)_2SO_4$	11.0
L-Tryptophan	8.2
L-Arginine	10.6
L-Methionine	2.4
DL-Alpha alanine	10.3
DL-Phenyl alanine	<1.0
L-Glutamic acid	12.1
L-Glutamine	11.9
Casein hydrolysate	9.3

[a] Cultivated in stationary liquid culture at 25° C for 16 days.

TABLE IV

THE EFFECT OF VARIOUS CARBON SOURCES UPON THE GROWTH OF CLADOCHYTRIUM REPLICATUM IN A MEDIUM CONTAINING NITROGEN IN THE FORM OF $(NH_4)_2SO_4$[a]

Carbon sources (2 g of carbon per liter of medium)	Dry weight in mg
Series 1	
None	<1.0
D-Glucose[b]	36.3
Gluconic acid	<1.0
Glucuronic acid[b]	<1.0
Saccharic acid	<1.0
D-Xylose[b]	<1.0
D-Fructose[b]	32.2
L-Sorbose[b]	<1.0
Sorbitol	<1.0
Sucrose[b]	<1.0
D-Lactose	18.9
Cellobiose	17.6
Starch	31.4
Series 2	
None	<1.0
D-Glucose[b]	33.0
Glycerol	1.1
D-Galactose[b]	5.2
L-Arabinose[b]	1.3
Dulcitol	<1.0
D-Mannose	34.2
Mannitol	<1.0
D-Melibiose	33.1
D-Raffinose[b]	<1.0
D-Trehalose[b]	<1.0
Sodium pyruvate	<1.0
Cellophane[c]	9.3

[a] Cultivated in shake culture at 21° C for 16 days.

[b] Compound sterilized by Seitz filtration. The other compounds were autoclaved.

[c] The value given is a minimal one since the fugus could not be separated from the cellophane upon which it was growing. The cultures were kept stationary in order to allow the fungus to become attached to the particulate substrate. The values were obtained by subtracting the weight of the cellophane plus the weight of the chytrid growing upon it from the weight of the cellophane dispensed into the medium.

that the optimal concentration of other compounds in terms of carbon is identical with that of glucose; however, it provided a reasonable basis for making comparisons. In all instances the carbon sources were sterilized separately and dispensed aseptically into the autoclaved components of the medium. The fungus grew vigorously with glucose, fructose, mannose, and melibiose and failed to metabolize the sugar acids that may be derived from glucose (TABLE IV).

DISCUSSION

Light is known to influence the synthesis of gamma carotene in the monocentric chytrid *Rhizophlyctis rosea* (de Bary & Woronin) Fischer (Haskins and Weston, **1950**). It did not, however, function in the formation of pigment by *C. replicatum*. This corroborates a report (Anonymous, **1958**) touching upon Willoughby's findings with eight strains of the fungus. In the present investigation nutrition and aeration rather than light were found to be the major factors which affect the development of pigment. The cultivation of the fungus in media containing yeast extract or tryptone limited its capacity to form the characteristic orange color. Perlman, *et al.* (**1946**) reported that the presence of inorganic impurities in another complex organic nutrient, molasses, inhibited the growth of a deuteromycete. A similar explanation may be applicable here. The presence of ions that are more toxic in the oxidized than in the reduced state may explain the scantier yields of *C. replicatum* in shaken as compared with stationary Medium A.

An interesting problem is presented by the failure of the chytrid to utilize raffinose, a trisaccharide in which galactose and fructose are joined to a centrally placed glucose unit. This triose may be considered as sucrose to which galactose is attached or as melibiose linked to fructose. Since this organism metabolizes melibiose and respires its constituent glucose and galactose units, it is curious that it does not assimilate raffinose. It may be inferred that the presence of the glucose-fructose bond which the fungus cannot rupture prevents the severing of the α-1,6-galactose-glucose link by the galactosidase that it does possess.

Although it appears probable that the improved yields made by the fungus with the galactose disaccharides, as contrasted with that obtained by the use of galactose proper, were due to the utilization of the glucose portion of the diose, this cannot be stated categorically. An alternative explanation is suggested by the work of Sistrom and Machlis (**1955**) with *Allomyces macrogynus* (Emerson) Emerson & Wilson. They found that the addition of small quantities of a readily respired compound may stimulate the utilization of one that is not respired appreciably by itself. Thus, the hydrolysis of the disaccharides, lactose and melibiose, would liberate glucose which may have accelerated the rate at which the galactose was metabolized.

The nutritional responses displayed by *C. replicatum* are consistent with the pattern currently (Cantino, **1955**; Cantino and Turian, **1959**) thought to characterize members of the Chytridiales. Thus the fungus (1) assimilated nitrogen in the form of inorganic salts, (2) required exogenously supplied thiamin, and (3) presumably metabolized inorganic

sulfur. This is the first report of a thiamin requirement for an inoperculate polycentric chytrid. Considered within the context of Crasemann's (**1954**) hypothesis that the progenitors of the Blastocladiales be sought among inoperculate thiamin deficient chytrids, the present finding may be of some phylogenetic significance. Caution is necessary, however, since studies of bacteria and higher fungi have shown that even different isolates of the same microorganism may vary with respect to vitamin requirements.

SUMMARY

A study of factors affecting the growth and pigmentation of *Cladochytrium replicatum* Karling revealed that it grew best from 15° to 25° C and in media having an initial pH of between 5.9 and 7.0. The fungus was dependent upon its environment for thiamin and the incorporation of 50 μg of that vitamin per liter of nutrient solution resulted in optimal harvests. Light was not found to influence yield or pigmentation whereas the fungus did develop coloration in those agitated media in which it grew vigorously. Under the latter conditions growth occurred in the form of orange star-shaped masses ranging from 2 to 6 mm in diameter, whereas the rhizomycelial mats formed in stationary culture did not become pigmented. *C. replicatum* assimilated nitrogen in the form of inorganic salts and gave optimal yields when supplied with glucose, fructose, mannose, or melibiose as carbon sources.

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NOTE ADDED IN PROOF: It has recently been reported that the pigment in this chytrid is lycopene (Fuller, M. S., and J. E. Tavares. **1960.** Biochimica et Biophysica Acta **44**: 589–590). This finding corroborates some of the results noted in the present study since it is known (Karrer, P., and E. Jucker. **1950.** Carotenoids. 384 p. Elsevier Publ. Co., New York) that lycopene synthesis may be independent of light whereas aeration is important.

CUCURBITARIA STAPHULA ON POPULUS AND ITS ASSOCIATION WITH MACROPHOMA TUMEFACIENS[1]

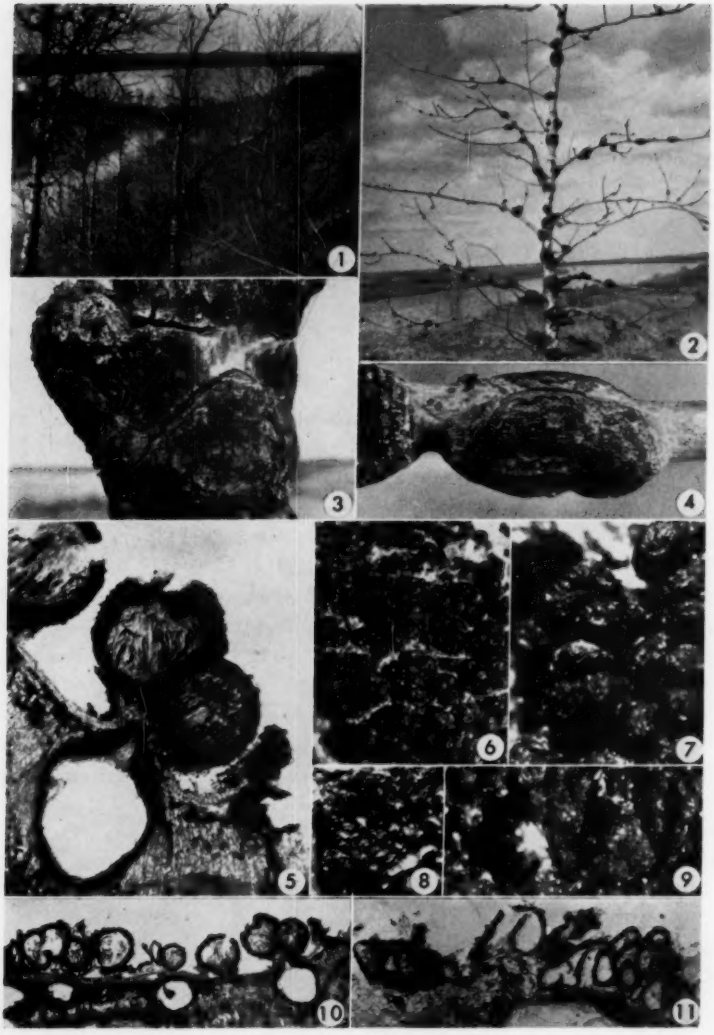
RUTH HORNER ARNOLD AND R. C. RUSSELL

(WITH 29 FIGURES)

Cucurbitaria staphula Dearness was first reported in 1926 in the Sixth Annual Report of the Prevalence of Plant Diseases in Canada (McCurry and Hicks **1926**, p. 32) as a fungus associated with a limb gall on *Populus balsamifera* L. at Buchanan, Saskatchewan. Since then it has been reported five times; four times in the Annual Report of the Canadian Plant Disease Survey (Conners **1929**, p. 64, Conners **1935**, p. 62, Conners **1939**, p. 93, and Conners & Savile **1944**, p. 98) associated with galls on limbs and trunks of *P. balsamifera* in Saskatchewan at Devil's Lake near Buchanan, Naisberry, Beaver Creek, Dana, and Indian Head, and a fifth time in "The fungi of Manitoba and Saskatchewan" (Bisby et al. **1938**, p. 52) as "*Cucurbitaria staphula* Dearness in manuscript" on galls on branches of *P. balsamifera* at Beaver Creek, Buchanan, Naisberry, and Pike Lake, Saskatchewan.

Although the name *Cucurbitaria staphula* Dearness was published six times, no diagnosis of the species was given in any of the publications cited above, and as far as can be ascertained, the fungus was never described by Dearness, as he intended. The Dearness Herbarium has now been made available for study and is housed, at present, in the Mycological Herbarium, Department of Agriculture, Ottawa, Canada, through the courtesy of the Montreal Botanic Garden to which it was bequeathed by Dearness. A type specimen for this species has been chosen from the material in the Dearness Herbarium labelled *Cucurbitaria staphula* by Dearness, and the species is described here in order to validate the name. Recent collections of this species have been made on *P. balsamifera* in the type locality and cultural studies of single ascospore and single conidial cultures of an associated conidial state belonging in the form genus *Pseudodichomera* have established the connection between the two states. Cultural studies of *Macrophoma tumefaciens*

[1] Contribution No. 94 from the Plant Research Institute, Research Branch, Canada Department of Agriculture, Ottawa, Ontario.



FIGS. 1–11. *Cucurbitaria staphula* on *Populus balsamifera*. FIG. 1. Diseased trees in type locality—Beaver Creek, Saskatoon, Sask. FIG. 2. One tree showing galls on branches and trunk covered with *C. staphula*. FIG. 3. Trunk gall × ½. FIG. 4. Branch gall × ½ showing crustose nature of perithecial stromata and concentric rings of perithecial projections on outer edges of galls. FIG. 5. Vertical section through perithecial cluster growing over old pycnidial locule of *Macrophoma tumefaciens* × 45. FIGS. 6, 7. Surface view of perithecial projections FIG. 6. × 10, FIG. 7.

Shear, a second conidial state associated consistently with *C. staphula* on the galls, showed *M. tumefaciens* (FIG. 27) to be a separate species. Dearness himself had noted the consistent association of these three fungi which he labelled *Cucurbitaria staphula*, *?Camarosporium*, and *Macrophoma tumefaciens*, on the galls on *P. balsamifera*.

Cucurbitaria staphula Dearness ex R. H. Arnold & R. C. Russell sp. nov. (FIGS. 1–17)

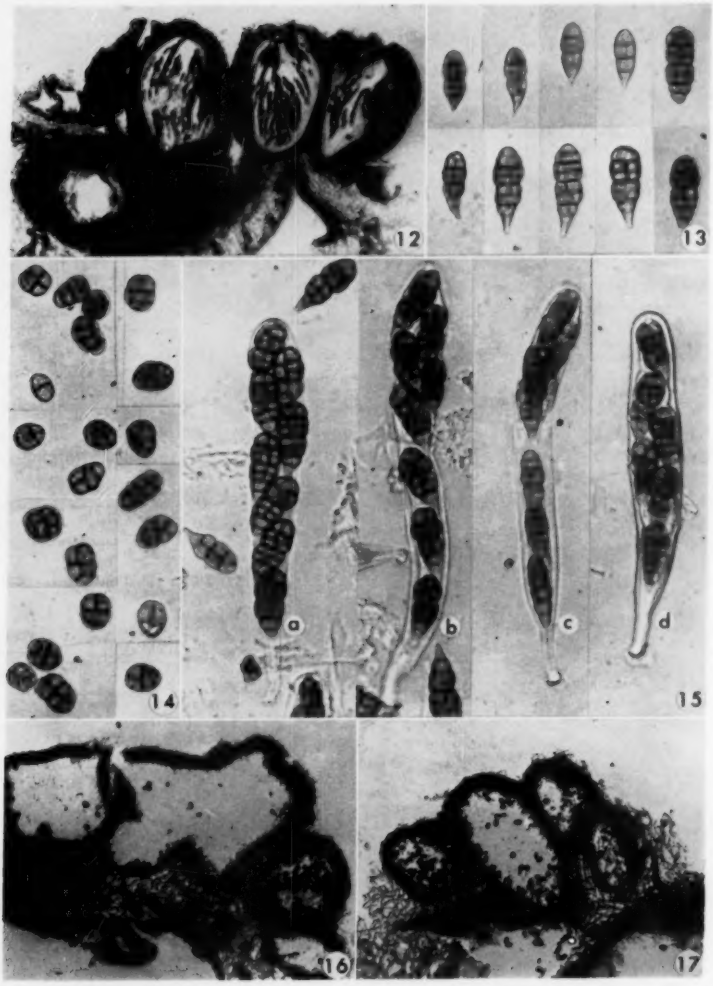
Loculi ascigeri intus eminentiis stromaticis, singuli in eminentia; eminentiae caespitosae crustam formantes, aut caespitosae in rimis corticeis tumoris, aut in annulis concentricis marginalibus tumoris. Eminentiae stromaticae perithecioideae a turbinatis subglobosae, atrae, rugulosae, plus minusve ad basas elongatae, connectae ad basas a strata pseudoparenchymatosa, ad summas in primis apiculatae ex ostiolo, deinde retusae quia ostiolum degenerat. Loculi ascigeri 335–470 × 165–300 μ, a turbinatis subglobosi. Asci 144–240 × 17.5–25.5 μ, clavati, substipitati, bitunicati, octospori. Ascospori 27.0–48.0 × 12.5–16.0 μ, oblique monostichi vel inaequaliter distichi, fulvi, muriformes, ovati, (3–)7-septati, circum septum principem conspicue constricti, parte summa 1–3-septata, saepius obtusa, rarius acuta, parte basa 1–3-septata, acuta vel caudata, interdum subacuta; quisque loculus semel vel saepius septulo longitudinali aut obliquo divisus. Pseudoparaphyses abundantes, articulati, septati, 1.5 μ lat. Status conidiophorus—*Pseudodichomera* sp. Loculi conidiali intus eminentiis stromaticis, singuli aut complures in eminentia; eminentiae caespitosae, crustam formantes aut caespitosae in rimis corticeis tumoris aut in annulis concentricis marginalibus tumoris. Eminentiae pycnidioideae ostiolatae deinde perforatae latissime. Loculi 100–300 × 75–295 μ, subglobosi, ovati, aut irregulares, solitarii vel aggregati in eminentia. Conidia 12.5–16.0 × 8.0–10.5 μ, fulva, muriformia, a subglobosis oblonga, 1–3-septata, septulis paucis longitudinalibus vel obliquis divisa, ad septa vix vel non constrictis, quidque conidium involutum velamine hyalino tenue, 0.5 μ crass. Conidia singula in conidiophoris 2.8–10.5 × 2.8–4.8 μ, brevibus, hyalinis, aut conidia sessilia.

Hab.: in *Populo balsamifera* L., *Populo tremuloidi* Michx., tumoris ramorum vel trunci uti *Macrophoma tumefaciens* Shear.

Cucurbitaria staphula differs from known species of *Cucurbitaria* in the size and shape of the ascospores and in its occurrence on galls on branches and trunks of *Populus* spp. in association with *Macrophoma tumefaciens* Shear.

Ascigerous state on the host: Perithecial locules in stromatic projections, one locule to a projection; projections closely clustered, crustose, or clustered in cracks in bark on galls or in concentric rings on the margins of galls or "rough bark" [a condition of the bark described

× 25. FIGS. 8, 9. Surface view of pycnidial projections FIG. 8. × 10, FIG. 9. × 25. FIG. 10. Vertical section of perithecial stromata on cortex of gall × 15. FIG. 11. Vertical section of conidial stromata on cortex of gall × 45.



FIGS. 12–17. *Cucurbitaria staphula* on *Populus balsamifera*. FIG. 12. Vertical section of perithecial stroma × 45. FIG. 13. Ascospores × 400. FIG. 14. Conidia × 400. FIG. 15. Asci × 400. (a) ascus before dehiscence of outer wall, (b, c) asci after dehiscence of outer wall in water mount, (d) ascus after dehiscence of outer wall and expulsion of two spores. FIGS. 16, 17. Vertical sections of conidial stromata × 80.

by Kaufert (1937)] on branches and trunks of *Populus* spp. in association with *Macrophoma tumefaciens*. Stromatic projections perithecium-like, turbinate to subspherical, black, externally roughened, slightly or strongly elongated at the base, the bases united by a thin pseudoparenchymatous layer consisting of brown hyphal cells mixed with cortical cells, each projection with a fine, black, papillate ostiolum when young but collapsing apically to appear flat on top and becoming recurved and perforate at maturity. Ascigerous locules 335–470 × 165–300 μ, subspherical to turbinate, lined with hyaline cells of stromatic tissue, hymenium covering base of locule. Asci 144–240 × 17.5–25.5 μ before extrusion of the inner ascus, bitunicate, clavate, substipitate to short-stipitate, eight-spored. Ascospores 27.0–48.0 × 12.5–16.0 μ, obliquely uniseriate or irregularly biseriate in the ascus, ovate, brown, muriform, 3–7-septate at maturity, constricted at all septa but strongly constricted at the primary septum; the upper half of the spore with 1–3 cross walls, usually obtuse at the tip, occasionally acute; the lower half of the spore with 1–3 cross walls, tip usually acute or caudate, occasionally subacute; the cells divided by one or more longitudinal or oblique cell walls. Ascospores surrounded by a thin hyaline sheath 0.5 μ thick, difficult to discern. Pseudoparaphyses, 1.5 μ wide, abundant, septate, one cell in thickness, joined by anastomoses.

According to a note on one of the packets in his herbarium, Dearness thought that the ascospores resembled a lead plumb-bob in shape and therefore gave this species the specific epithet *staphula* from the Greek σταφύλη.

Conidial state on the host: Conidia produced in locules in black stromatic projections, one or more locules in one stromatic projection; projections closely aggregated on a thin basal stroma to form crust-like layers in cracks on the surface of galls, or projections occurring singly or in small groups in concentric rings on the margins of galls or "rough bark" on branches and trunks of *Populus* spp. in association with *Macrophoma tumefaciens;* projections prominent, pycnidium-like, at first ostiolate but becoming widely perforate with age, exterior roughened. Locules 100–300 × 75–295 μ, subspherical to ovoid or irregular in shape, crowded if more than one in a projection. Conidia 12.5–16.0 × 8.0–10.5 μ, dark brown, muriform, subspherical to irregularly oblong in shape, sometimes tapered at one end; with 1–3 cross walls and few longitudinal or oblique walls, slightly or not constricted at the septa, surrounded by a thin hyaline sheath 0.5 μ thick. Conidia borne singly on the tips of short, hyaline, simple conidiophores 2.8–10.5 × 2.8–4.8 μ arising from the cells lining the locules, or conidia sessile, borne directly from the stromatic cells lining the locules.

In older pycnidial locules, hyphal pegs were seen extending out into the locules from the stromatal cells lining the locules. These hyphal pegs were simple, non-septate or septate, filiform, up to 40 μ long and 2.5–3.0 μ wide, and strongly resembled conidiophores. However, no conidia could be found attached to them and they appeared to be extensions of the short conidiophores after the conidia had abscissed.

The conidial state belongs in the form genus *Pseudodichomera* v. Höhnel.

A few pycnidial locules containing hyaline, allantoid to ovoid conidia 2.0 × 0.5 μ, borne singly on the tips of filiform, non-septate conidiophores, were found in the same stromata as the locules containing the *Pseudodichomera* state. These may represent a second conidial form of *Cucurbitaria staphula* but they have not been observed in the single-spore cultures on two per cent potato dextrose agar and therefore the connection cannot be considered established.

HOSTS: *Populus balsamifera* L., *Populus tremuloides* Michx.

DISTRIBUTION: Saskatchewan, Canada—Beaver Creek near Saskatoon, Candle Lake, Devil's Lake near Buchanan, Indian Head, Naisberry, Pike Lake, Redberry.

SPECIMENS EXAMINED:[2] J. Dearness Herbarium no. 6104—(TYPE) Beaver Creek, Saskatoon, Sask., *Russell* (R25), Sept. 7, 1929; Buchanan, Sask., *Russell,* Oct. 29, 1924; Naisberry, Sask., *Russell,* Sept. 2, 1927; Beaver Creek, Saskatoon, Sask., *Russell,* July 28, 1928; Saskatoon, Sask., comm. *Russell,* May 31, 1929; Pike Lake, Sask., *Russell* (R34), Oct. 6, 1929. Beaver Creek, Saskatoon—DAOM 14544, *Russell* (R25), Sept. 7, 1929 (ISOTYPE); DAOM 63181, *Russell,* July 28, 1928; DAOM 5007, *Güssow et al.* (S530), Sept. 3, 1937; DAOM 64002, *Bier & Russell* (S689), Aug. 5, 1938; DAOM 59082, *Russell & Riley* (S1538), April 7, 1958; DAOM 59083, *Russell* (S1540), April 13, 1958; DAOM 59081, *Russell & Cormack* (S1544), May 19, 1958. Buchanan—DAOM 63179, *Russell,* Aug. 3, 1926; DAOM 64001, *Russell & Mead* (S15), Aug. 19, 1929. Candle Lake—DAOM 64003, *Zalasky & Riley* (SAFB 1425), Sept. 3, 1958. Indian Head—DAOM 63180, *Russell* (S1285), Sept. 20, 1943. Redberry—DAOM 64004, *Zalasky & Russell* (SAFB 1426), June 12, 1959.

The distribution records listed above show that *Cucurbitaria staphula* has been recorded, to date, only from central Saskatchewan. It has been found only on galls and "rough bark" on branches and trunks of

[2] The numbers in brackets preceded by R and S are collection numbers of R. C. Russell, Saskatoon. DAOM and SAFB are abbreviations for Herbaria listed in Index Herbariorum (1959).

P. balsamifera and *P. tremuloides*, associated with *Macrophoma tumefaciens*, and nearly all collections have been made by the junior author and colleagues.

In the reports in the Canadian Plant Disease Survey and in correspondence contained in the Dearness Herbarium, the occurrence of the galls was described as moderate to abundant, the number of trees affected at any one location varying from a few to more than one hundred. Russell made the first collection of *C. staphula* at Buchanan in 1924 and it was reported to Dearness at that time that the galls were present in thousands on the poplars and seemed to be killing them. In 1929 (Conners **1929**, p. 64) Russell reported that he had revisited the site of the original collection and found that many of the trees that were then infected were still living although some badly infected branches on them were dead. A similar disease on the bark of the trunks was also observed. He reported that a high percentage of the trees in one locality at Beaver Creek were more or less heavily infected and that most of the infected limbs were dead, some were dying, and a few were still green. Later collections made by Russell at the Beaver Creek locality in 1958 included galls on both living and dead branches.

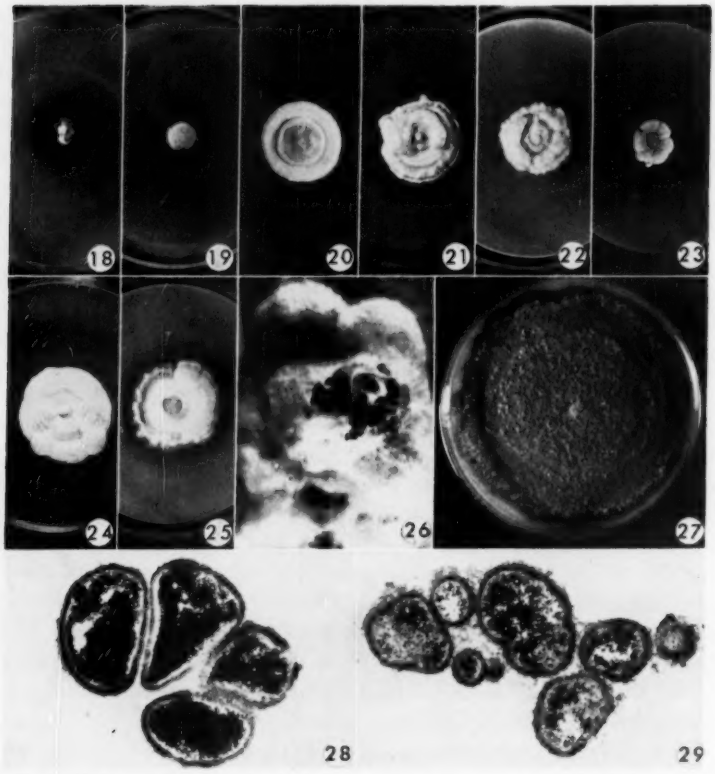
Cultures on two per cent potato dextrose agar: The following description is based on sixty single ascospore and single conidial cultures obtained from DAOM 59081 and DAOM 59082 (FIGS. 18–26, 28–29).

(a) Cultures grown in petri plates at room temperature (approximately 21° C) in diffuse light.

Growth Rate: slow; radial growth of colony 4–15 mm in 8 weeks.

Macroscopic Characters: Advancing edge—not differentiated from aerial mat, or sharply delimited from it, a narrow zone of closely woven, appressed or submerged mycelium, white or tan in color. Aerial mat—at first compactly woven, hemispherical, white with a smooth surface; by three weeks becoming tinged with gray, gray-brown, or tan [Drab, Grayish Olive, Smoke Gray, Deep Olive Gray][3], surface smooth, velvety, some colonies with a sharply depressed zone around central part of mat; colors deepened in older colonies and new growth on some zonate with a sharply defined, narrow, appressed zone at even or fluted edge. Reverse side—at first white, even, beneath mat; then submerged mycelium forming a compactly woven hemispherical mass in agar, and colors of surface mat seen through agar, gray or tan with a white edge [Tawny Olive, Saccardo's Umber]; by three weeks most colonies with tan aerial mycelium surrounded by a brown stain [Cinnamon Buff, Grayish Olive,

[3] All colors cited in brackets throughout description are those of Ridgway (**1912**).



FIGS. 18–26. *Cucurbitaria staphula*—single ascospore cultures grown on two per cent potato dextrose agar. FIGS. 18–25. Cultures grown in petri plates for eight weeks at room temperature × ½. FIG. 26. Culture grown in test tube for eight weeks at room temperature and eight weeks at 15° C, showing black conidial spore tendrils oozing from pycnidial locules × 2. FIG. 27. *Macrophoma tumefaciens*—single conidial culture grown on two per cent potato dextrose agar for four weeks at room temperature × ½. FIGS. 28, 29. *C. staphula*—vertical sections of conidial stromata produced in single ascospore culture on two per cent potato dextrose agar × 20. FIG. 28. Stroma from culture grown in test tube. FIG. 29. Submerged stroma from culture grown in petri plate.

Light Grayish Olive] in agar extending approximately 4 mm beyond edge of colony; by eight weeks, tan or orange beneath the whole mat or shades of tan and gray-brown in zones [Tawny Olive, Avellaneous, Sayal Brown, Cinnamon Buff, Clove Brown, Olive Brown, Bister], brown stain [Tawny Olive] associated with some colonies extending 10–15 mm into agar beyond edge of colony and gradually diffusing into agar.

Microscopic Characters: Mycelium composed of thick-walled hyphae, 2.5–3.5 μ diam, sparingly septate, and branched at right angles to the hyphae; submerged mycelium loosely interwoven and mycelium of aerial and surface mats closely interwoven, the mycelium of the surface mat with numerous, short, coiled branches; at nine weeks conidial stromata found within the aerial mat with mature conidia typical of those found in nature; stromatic areas occurred in zones where the hyphae were more closely septate with the majority of the cells swollen, subspherical, 4.5–14.0 μ diam; in areas where conidial locules occurred, swellings very numerous, and mat in section a mass of irregularly spherical, closely-packed cells; in a spore producing area, walls and contents of these cells dark brown to black and more compressed forming a dark stromatic layer within which the cells remain hyaline, and from these hyaline cells the conidia arise directly without conidiophores; as more conidia are formed these cells disintegrate, eventually leaving a locule filled with conidia; locules globose to irregular in shape, 200–600 × 200–475 μ; conidia same size, shape and septation as those on the host. A few irregularly shaped conidia, e.g. two conidia joined at the base, seen.

(b) Cultures grown in tubes—grown in diffuse light at room temperature for 8 weeks, then at 15° C.

Macroscopic characters of colonies similar to those in petri plates but stain in agar darker in color. Microscopic characters the same as for colonies in petri plates but in addition to the stromatic layer produced within the aerial mat at nine weeks, black stromata with pycnidial projections and mature conidia oozing from the ostioles were found on the surface of the aerial mat at four months in some cultures. These stromata 2–7 mm diam, irregularly circular in outline, similar in microscopic structure to the submerged conidial stromata, composed of subspherical to irregularly shaped, closely packed cells and consisting of an outer layer 24–40 μ thick composed of cells with dark brown walls and pale brown contents and a central portion of hyaline cells, within which locules 160–370 μ diam arose; locules globose to irregular in shape, some coalescing within the stroma to form a large irregular chamber; conidia borne directly on hyaline stromatal cells lining the locules, not from conidiophores as in nature; conidia of the same shape, septation, and color as in nature, but slightly larger, 12.0–21.6 × 9.6–12.8 μ.

ASSOCIATION OF CUCURBITARIA STAPHULA WITH MACROPHOMA TUMEFACIENS

The relation of *C. staphula* to the galls on which it is found is not clear. These galls on *Populus* closely resemble those described on *Populus trichocarpa* Torrey & Gray in Montana by Hubert (**1915**) associated with *Macrophoma tumefaciens*. The suspected pathogenicity

of *M. tumefaciens* was supported by histological work by Hyde (**1922**) and this species was proved capable of producing galls on aspen by Kaufert (**1937**) through artificial inoculation studies. Kaufert reported branch galls and "rough bark" of trunks caused by *M. tumefaciens* to be common on aspen in the Lake States, especially on those trees on poor sites.

M. tumefaciens has been reported in Canada on galls on *P. balsamifera* at Edmonton, Alberta (Conners **1939**, p. 93), on *P. trichocarpa* in the Quesnel district of British Columbia (Conners & Savile **1949**, p. 99), on *Populus* sp. at Kenora, Ontario (Conners & Savile **1949**, p. 99), and on *P. tremuloides* at Humboldt, Saskatchewan as *"Dothiorella gallicola* Dearn. in litt." (Bisby et al. **1929**, p. 64). Canadian specimens of *M. tumefaciens* have been examined in the Dearness Herbarium on *Populus* sp. from Bird's Hill, Manitoba (Bisby 4850), and on *P. tremuloides* from Humboldt, Sask., sub *"Dothiorella gallicola* Dearn. n.s." (Dearness 6769); and in the Mycological Herbarium, Department of Agriculture, Ottawa, on *Populus* sp., Renfrew County, Ont. (DAOM 63132), on *Populus* sp. at Kenora, Ont. (DAOM 6693), on *P. balsamifera*, Doré Lake, Sask. (DAOM 56739), on *P. tremuloides* at Red Deer, Alta. (DAOM 46807 & DAOM 56810), and on *P. trichocarpa* at Bella Coola, B. C. (DAOM 67943). In addition to these specimens, *M. tumefaciens* has been found on *P. balsamifera* and *P. tremuloides* in Saskatchewan, in all except one of the collections cited above for *Cucurbitaria staphula*, on young galls and on the edges of old galls where the tissue of the host was most recently affected. The one exception was a collection in the Dearness Herbarium from Naisberry which contained no young material and was in poor condition.

In view of the occurrence of *M. tumefaciens* on young galls and on younger portions of old galls in the collections of *C. staphula*, and the widespread distribution of *M. tumefaciens* in Canada, it appears that the *Cucurbitaria* is a secondary invader in the tissue of galls initiated by *M. tumefaciens*. However, the exact relation of *C. staphula* to the galls can be determined only by artificial inoculation studies. It is possible that *C. staphula* is responsible for increasing the size of galls already initiated by *M. tumefaciens* or that *C. staphula*, itself, is capable of inducing gall formation on *Populus* species.

NOTES ON THE GENUS PSEUDODICHOMERA

Some problems in nomenclature have been encountered in deciding on a form genus for the conidial state of *Cucurbitaria staphula* and it is thought advisable to include here the information gleaned in this study

concerning the genus *Pseudodichomera*. Von Höhnel (**1918**) segregated this genus from *Dichomera* Cooke sensu Sacc. (Cooke **1878**, p. 24, Saccardo **1880**, p. 8) to include conidial states of *Cucurbitaria* species on the basis that these forms have a botryoidal stroma and pycnidia joined at the base by a stromatic layer. At the same time he emended the genus *Dichomera* to include only those species with a flat cushion-shaped dothideaceous stroma with the locules completely immersed below the surface in a single row. In doing this, von Höhnel caused some confusion by designating as type species of the genus *Pseudodichomera*, *P. varia* (Pers.?) v. Höhn., with the basionym ?*Sphaeria varia* Persoon, and synonyms *Camarosporium varium* (P.?) Starbäck and *Dichomera varia* (P.?) Diedicke. From a study of the literature involved (Persoon **1801**, p. 52, n. 97, Fries **1823**, p. 416, n. 213, Starbäck **1894**, pp. 89–90, Diedicke **1915**, p. 687, and von Höhnel **1918**, pp. 185–188) it becomes clear that the basionym for the type species should not be ?*Sphaeria varia* Persoon but *Camarosporium varium* Starbäck which was described by Starbäck (**1894**) from a specimen he found in Fries' herbarium labelled "*Sphaeria varia* Pers. syn. p. 52?". Starbäck states (**1894**) that it is doubtful if this specimen in Fries' herbarium, collected and determined by Fries, is the same as *Sphaeria varia* Pers., i.e., he considers the Fries specimen a possible misidentification. Thus, with present evidence, the nomenclature of the type species of the genus *Pseudodichomera* should read as follows:

PSEUDODICHOMERA VARIA (Starb.) von Höhnel
as "*P. varia* (Pers.?) v. H." 1918. Hedwigia **60**: 187.
≡ *Camarosporium varium* Starb.
as "*C. varium* (Pers.? Fr.)" 1894. Bihang till K. Svenska Vet.-Akad. Handlinger. Band 19. Afd. III, No. 2: 89–90.
≡ *Dichomera varia* (Starb.) Diedicke
as "*D. varia* (Pers.?) Died." 1915. Kryptogamenflora der Mark Brandenburg. vol. 9: 687.

and the type specimen would then be Fries' specimen, sub *Sphaeria varia* Pers.

To complicate matters further, there is no material under the name *Pseudodichomera* either in the von Höhnel herbarium contained in the Farlow Herbarium at Cambridge, Mass., or among the von Höhnel specimens at Kew. A specimen labelled "*Dichomera Laburni* Cooke et Mass.", one of the three species transferred to *Pseudodichomera* by von Höhnel (**1918**), was located in the von Höhnel herbarium contained in the Farlow Herbarium.

In this study, the following exsiccati specimens have been examined:

Rabenh.-Winter, F. europ. 3781 sub "*Dichomera Elaeagni* Karsten" transferred to *Pseudodichomera* and cited by von Höhnel (**1918**).

v. Höhn. herb., "Sphps. 2553a" [scr. v. Höhnel] sub "*Dichomera Laburni* Cooke et Mass." transferred to *Pseudodichomera* by von Höhnel (**1918**).

Petrak, Myc. Gen. 1516 sub "*Camarosporium Laburni* (West.) Sacc." transferred to *Pseudodichomera* by von Höhnel (**1918**).

Roumeg., F. gall. exsicc. Nr. 2290 sub "*Staurosphaeria Tiliae* West." cited by von Höhnel (**1918**) as an example of a true *Dichomera* sensu v. Höhnel.

The specimens cited above which were transferred to *Pseudodichomera* by von Höhnel agree with von Höhnel's description of *Pseudodichomera* except that the pycnidia are in reality stromatal projections with one or more pycnidial locules in each projection and joined at the base by a stroma, and the conidiophores were found to be inconspicuous and variable in size but very short and simple when present, or lacking altogether. The conidial state of *Cucurbitaria staphula* Dearn. ex R. H. Arnold & R. C. Russell is the same as these species in structure. As described above, hyphal pegs resembling conidiophores were found growing out into the older pycnidial locules of *C. staphula*. Von Höhnel may have been referring to similar structures when he described simple, medium-length conidiophores for *Pseudodichomera*. No authentic specimens of *Camarosporium varium* Starb. in good fruit have been examined but Starbäck's description and illustration of this species (Starbäck **1894**) agree with von Höhnel's description of the genus *Pseudodichomera* except that Starbäck did not mention or illustrate the conidiophores.

The specimen, Roumeg. F. gall. exsicc. Nr. 2290, cited by von Höhnel as an example of a true *Dichomera*, fits von Höhnel's emended description of the genus *Dichomera*. It has small, flat pulvinate, black stromata, barely erumpent through the bark, with pycnidial locules completely immersed in the stroma and visible externally only as very slight projections on the surface of the stroma with an inconspicuous papilliform ostiole in the center of each projection. The conidia are borne singly and terminally on prominent, hyaline, simple conidiophores, 2–3 times the length of a conidium, lining the locules.

Thus, according to the information at hand, the form genus *Pseudodichomera* v. Höhnel differs from the form genus *Dichomera* Cooke sensu Sacc. emend. v. Höhnel not only in the form of the stroma but also in the type of conidiophore, and is further distinguished by its

genetic connection with *Cucurbitaria* species. For these reasons it is retained here for the form genus of the conidial state of *Cucurbitaria staphula*. Welch (1926) also accepted the form genus *Pseudodichomera* v. Höhnel for the conidial state of *Cucurbitaria* species. However, it is doubtful if von Höhnel examined the type specimen of *Dichomera* Cooke sensu Sacc. and after a survey of the literature in the course of this study, it has become clear that an examination of type specimens and a critical review of the *Dichomera-Pseudodichomera-Camarosporium-Cellulosporium* complex is needed in order to set generic limits more exactly in this group of fungi imperfecti with dark muriform conidia.

ABSTRACT

Cucurbitaria staphula Dearness ex R. H. Arnold & R. C. Russell sp. nov. is described and the previously published name, *Cucurbitaria staphula* Dearness, thus made valid. *C. staphula* is found in Saskatchewan, Canada, on galls on *Populus balsamifera* L. and *Populus tremuloides* Michx., in association with *Macrophoma tumefaciens* Shear. The genetic connection of a conidial state of *C. staphula* belonging in the form genus *Pseudodichomera* von Höhnel has been established by cultural methods and a short discussion of the nomenclature and taxonomy of *Pseudodichomera* is given.

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NOTES AND BRIEF ARTICLES

Observations on Coprinus ephemerus

Coprinus ephemerus Bull. ex Fr. is normally a bipolar species. Miss Mounce, while acknowledging Brefeld's claim of monospore fruiting bodies, was unable to produce these herself (1921. Brit. Myc. Soc. Trans. **7**: 198–217). In the normal cross, the basidia of the resulting fruit bodies possess four sterigmata and four spores, two spores of each mating type. However, monospore fruiting bodies have been observed many times in the strains used here. The basidia of these fruit bodies possess only two sterigmata, with two spores, both of one mating type. The spores are often aberrant in size and shape or both. Some question is thrown on Sass's proposal of a separate form *bisporus* (1929. Amer. J. Bot. **16**: 663–701), since it now seems likely that this form was merely an isolate of one mating type of this normally bipolar species.

Spore germination experiments were performed with the normal spores of *C. ephemerus* (both mating types being used). Two media were used successfully, Fries's (1956. Physiol. Plant. **6**: 551–563), and Madelin's (1956. Ann. Bot. n.s. **20**: 307). In both cases, the absence of the respective amino acid and vitamin additives had no effect on spore germination percentage. However, asparagine is required for growth, and thiamin for fruiting. Substitution of $NaNO_3$ and NH_4NO_3 for ammonium tartrate in Fries's medium inhibited germination slightly but not significantly, but the addition of NH_4NO_3 to Madelin's medium raised the percentage of spore germination considerably.

Three germination aids were employed. No increase in germination percentage was obtained when sodium acetate or sodium hydroxide were used, either in the media or as spore suspension solutions. Furfural added to the media at 1/1000, raised spore germination from 40% to 85%, while the soaking of spores for one-half hour in a 1/10,000 solution in water raised germination to 91%.

Oidia were produced in variable numbers on malt extract-yeast agar. A conidial suspension from one ten-day-old colony in ten ml water reached as high as 4×10^6 spores/ml.—Ronald H. Petersen, Columbia University, New York City 27, N. Y.

CERCOSPORA LEAFSPOT OF LUFFA ACUTANGULA

Ribbed gourd (*Luffa acutangula* Roxb.) is one of the common cucurbitaceous vegetables cultivated in the tropical and subtropical regions. During the rainy season, starting from September to February–March, a severe leafspot disease of the crop is commonly found in many places in and around Annamalainagar, Madras State, India. The disease first manifests on the leaves in the form of specks or stray spots and, with the increase in atmospheric humidity during the northeast monsoon, spreads rapidly. Under favorable weather conditions the disease becomes severe affecting most of the leaves and causing shot-hole and withering of the leaves (FIG. 1). Due to the disease, growth of

FIG. 1. Leafspots caused by *Cercospora annamalaiensis* on *Luffa acutangula*.

plant is checked and the yield is severely affected. The plants rarely flower and fruits, if set, are very much reduced in size. We found the disease to be caused by a species of *Cercospora*.

Hara (1) recorded *C. luffae* Hara on *L. acutangula*. This has subsequently been included tentatively under *C. citrullina* Cooke by Chupp (2). The fungus under study is quite distinct from *C. citrullina* in several respects and so is described and named. Several attempts to culture the fungus failed, whereas *C. citrullina* occurring on other cucurbitaceous hosts could easily be cultured. The isolates of *C. citrullina* from bitter gourd (*Momordica charantia* L.), snake gourd (*Trichosanthes anguina* L.), and bottle gourd (*Lagenaria vulgaris* Ser.), when inoculated on ribbed gourd failed to infect. The details of these studies will be published elsewhere.

The disease first manifests as a minute dull white speck with reddish brown margin and pale yellow halo. Soon it increases in size resulting in a leafspot, 0.5 to 7 mm in diameter, circular to irregular, membranous, bright white, with greyish margin on the upper surface and dull white with tannish grey margin on the lower surface. The white portions of the spot soon dry and fall away from the leaf, resulting in shot-holes. There may be a few or many spots on the leaf; older leaves are more commonly affected than the younger ones. The spots are usually distributed separately throughout the leaf blade, but with increase in the disease intensity they may coalesce, causing large patches which cover

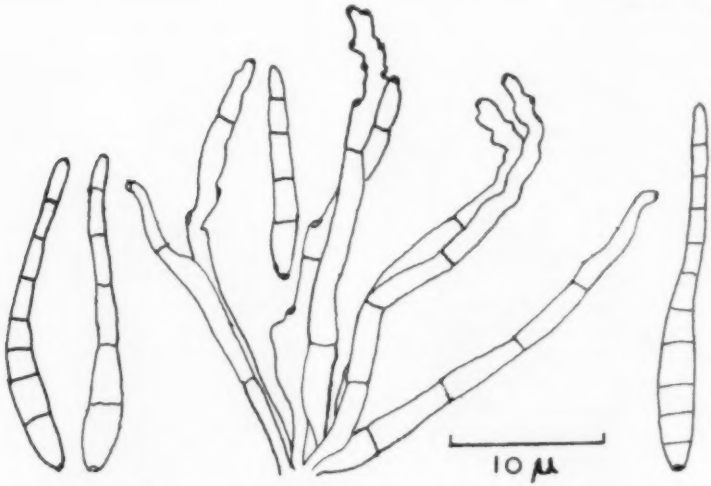

FIG. 2. Conidiophores and conidia of *Cercospora annamalaiensis*.

major portions of the blade. These patches turn dark brown and dry, the white portions fall off, leaving shot-holes with dark tan margins.

The hyphae are intercellular, septate, branched and orange colored. They form a thick layer of stroma immediately below the epidermis. The fascicles arise from the stroma in loose clusters and emerge through stomata or epidermis. The conidiophores are in clusters of 7 to 10, or more, 2.9 to 4.4 μ in diameter at their thickest point and 20.3 to 110.2 μ in length, reddish brown in mass and orange brown singly, tortuous, with prominent spore scars 3 to 6 on each conidiophore, with truncate or subtruncate tips, sparsely 2–5-septate, and the cell walls darker colored (FIG. 2).

Conidia are narrowly obclavate, hyaline, thin walled, straight or

slightly curved, septate with 4 to 13 distinct septa, base obconically truncate, tip subacute, conidia 2.9 to 4.4 μ in breadth at the thickest portion and 23.2 to 130.5 μ in length (FIG. 2).

Cercospora annamalaiensis Rang. & Chandr., sp. nov.

Cum primo apparet, puncta minuta efformat obscurata alba marginibus rubro-brunneis et corona pallide lutea; paulo post augetur in foliorum maculas 0.5–7 mm diam, circulares vel irregulares, membranaceas, lucide albas, marginibus griseis in pagina superiore et obscurate albis et margine alutaceo-griseo in inferiore pagina. Partes albidae macularum cito desiccantur atque decidunt, efformatis cavis glandis bellicae similibus. Maculae in singulis foliis nonnullae vel plurimae; folia vetustiora saepius infecta novellis. Maculae vulgo inter se distinguuntur distributae per totam faciem folii, sed infectione intensiore evadente convenire et coalescere possunt, maculis multo maioribus efformatis quae totam fere paginam folii tegunt. Hae vero maculae evadunt fusce brunneae et siccae, partibus albis decidentibus cavis efformatis margine alutaceo ornatis.

Hyphae inter cellulas hospitis plantae adsunt, suntque septatae, ramosae et aurantiacae, immediate sub epidermidem coactae efformato crasso stromate. Fasciculi e stromate surgentes emergunt laxe aggregati per stomata vel epidermidem. Conidiophori aggregati 7–10 vel plures, 2.9–4.4 μ diam. ad partem crassissimam, 20.3–110.2 μ longi, rubro-brunnei in massa, singuli vero aurantiaco-brunnei, flexuosi, cicatricibus sporarum tribus ad 6 in unoquoque conidiophoro eminentibus, apicibus truncatis vel subtruncatis, septis 2–5 distinctis et cellularum parietibus obscurioribus.

Conidia anguste obclavata, hyalina, parietibus tenuibus praedita, recta vel tenuiter curvata, septata 4–13 septis distinctis, basi obconice truncata, apicibus subacutis, 2.9–4.4 μ lata ad partem crassissimam, 23.2–130.5 μ longa.

Typus lectus in foliis viventibus *Luffae acutangulae* Roxb. ad Annamalainagar, in statu Madras, die 15 martii anni 1958 a S. Chandrasekaran et positus in Herb. Crypt. Ind. Orient (HCIO) in urbe New Delhi.

On the living leaves of *Luffa acutangula* Roxb., ribbed gourd, Annamalainagar, Madras State, March 15, 1958, *S. Chandrasekaran*. TYPE specimen deposited in Herb. Crypt. Ind. Orient (HCIO), New Delhi.

The authors are thankful to Dr. Charles Chupp for his expert advice on the systematics of the *Cercospora* and also to Rev. Dr. H. Santapau for the Latin translation of the new species.—G. RANGASWAMI AND S. CHANDRASEKARAN, Department of Agriculture, Annamalai University, Annamalainagar, Madras, India.

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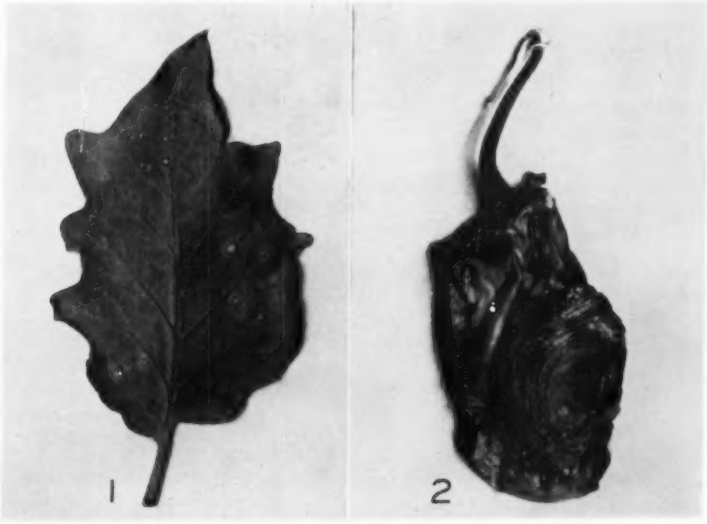
ALTERNARIA MELONGENAE CAUSING LEAFSPOT AND FRUIT SCAB OF EGGPLANT AND FRUIT ROT OF CHILI

Alternaria leafspots of solanaceous plants are common in several countries. *Alternaria solani* (Ell. & Mart.) Jones & Grout has been reported to cause leafspot of eggplant (*Solanum melongena* L.) in the U.S.A., U.S.S.R., Bolivia, Australia, Canada, and other countries (Neergaard, **1945**). Pucci (**1948**) reported that eggplants in Argentina were severely attacked by a species of *Alternaria,* which was identical with *A. tenuis* f. *genuina* Una. In India Vasudeva (**1954**) reported that an *Alternaria* sp. isolated from eggplant leafspot was morphologically similar to *A. tenuis* Nees., but was different in its host range.



FIGS. 1, 2. *Alternaria* on *Solanum melongena.* FIG. 1. Leafspots. FIG. 2. Fruit scab.

During November 1958 to February 1959 and again from October onwards, a severe leafspot and fruit scab of eggplant was noticed in Annamalainagar and its neighborhood of South Arcot District, Madras State, India. The leafspot infection first appeared as a light brown speck about 2 mm, which when fully developed, was about 5 mm, circular, light brown, and bound by a dark brown peripheral ring (FIG. 1). The disease appeared mainly on mature leaves, twenty spots or less on each leaf. Shot-hole symptoms also commonly appeared. In the field

fully mature eggplant fruits commonly were affected by the fungus. The lesions first appeared as brown, circular, flat spots about 4 mm. When fully developed the lesions were in the form of large scabs, circular or elliptical, 2 to 4 cm broad (FIG. 2). The scabs were considerably sunken; the depression sometimes extending 7 or 8 mm in the centre. The affected skin is leathery, the flesh below hard and discolored dirty brown to a depth of 1 cm. In some fruits the infection extended to the seeds, which were shrivelled and dark colored. The same fungus also caused fruit rot of chili or red pepper (*Capsicum annuum* L.) in the fields during November–December 1958, affecting

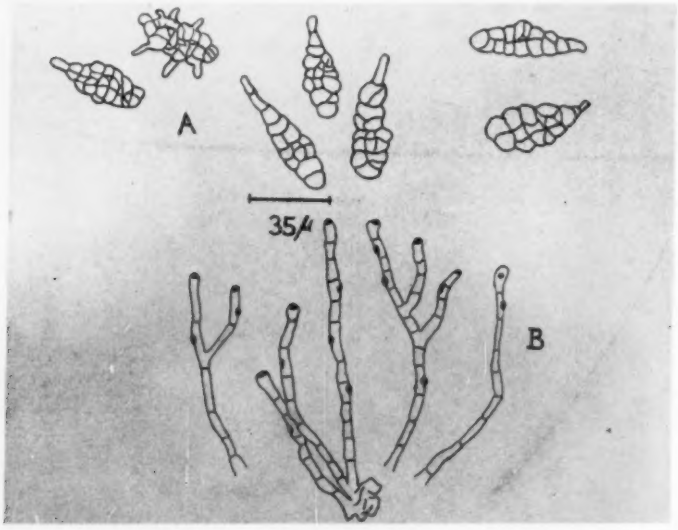


FIG. 3. A. Conidia of *Alternaria melongenae*. B. Conidiophores of *Alternaria melongenae*.

mainly the red ripe fruits, but not the green ones. Large irregularly elliptical or oval patches of water-soaked and gray rotting tissues were found on the surface of the fruits, more commonly on the portions exposed to the exterior than on the side facing the plant stem. The three diseases were found to be caused by a species of *Alternaria*. The causal organism was cultured, its pathogenicity established, and its cultural and physiological properties compared with those of five other isolates of *Alternaria* occurring on solanaceous hosts in this tract. The fungus causing the three disease symptoms is described as a new species.

Alternaria melongenae Rangaswami and Sambandam, sp. nov.

Maculae pallide brunneas, circulares, diametientes 2–5 mm, marginibus brunneas fusco, nonnumquam efformatis cavis glandis bellicae similibus. Mycelium intercellulare et intracellulare, hyalinum vel pallide brunneas, diametientes 3–6 μ, conidiophori amphigeni, emergentes 2–3 per stomata, ad epidermiden, septati, pallide brunneas, recti vel geniculati, 65–91 × 5–7 μ. Conidia usque penterna in catenulas rectas disposita brunneas, obclavata, muriformia, septis transveris 4–7, verticalibus vel obliquis 0–5 ornata, ad septa singula depressa, laevia, 28–84 × 7–17.5 μ, rostrate, rostris pallide brunneas, 10.5–52.5 μ (FIG. 3).

Typus lectus in foliis viventibus *Solanum melongena* L. ad Annamalainagar, in Statu Madras, mensu decemberi 1958 a C. N. Sambandam, et positus in Herb. Dept. Agri., Annamalai University, Annamalainagar, Madras, ed in Herb. Crypt. India Orient (HCIO), New Delhi (Accessionis No. 26,663).

Spots light brown, circular, 2–5 mm with dark brown margin, sometimes resulting in shot-holes. Mycelium both intercellular and intracellular, hyaline to light brown, 3–6 μ in diameter. Conidiophores amphigenous, arising in fascicles of 2–3 through the stomata and epidermis, septate, light brown, straight or geniculate 65–91 × 5–7 μ. Conidia in straight chains of 2–5, brown, obclavate, muriform, with 4–7 cross septa, 0–5 vertical septa and depressions along the septations, smooth surfaced, 28–84 × 7–17.5 μ, beaked, beaks light brown and 10.5–52.5 μ in length (FIG. 3).

On the living leaves of *Solanum melongena* L., Annamalainagar in Madras State, December 1958, *C. N. Sambandam*, TYPE in Herbarium of the Department of Agriculture, Annamalai University, Annamalainagar, Madras, and in Herb. Crypt. India Orient (HCIO), New Delhi (Accession No. 26,663).

The fungus is distinct in certain morphological characters and in producing profuse soluble purple pigment in synthetic as well as in complex agar and liquid media. Among the four known chromogenic species of *Alternaria*, *A. solani*, *A. linicola* Neerg., and *A. anagallidis* Raabe do not readily sporulate in culture media, while *A. porri* (Ell.) Neerg. may produce a very limited number of spores (Neergaard, **1945**). *A. melongenae* sporulated profusely in all the media tested, even after repeated subculturing for over 15 months. In the host range studies it was found to infect chili leaves and fruits, and leaves of tomato and *Solanum nigrum* L., besides leaves and fruits of eggplant, but failed to infect several other solanaceous leaves including those of *Datura* spp., potato, tobacco and nonsolanaceous hosts. Rangaswami and Rao (**1957**) reported that *A. cyamopsidis* Rang. & Rao from *Cyamopsis tetragonoloba* Taub. could infect, to some extent, eggplant leaves on artificial inoculation, but *A. melongenae* differed considerably from *A. cyamopsidis* in

its morphological, cultural and physiological properties, and in its host range.

A detailed report on the comparison of the morphological, cultural, and physiological properties, as well as the host range of *A. melongenae* and five other isolates of *Alternaria* spp. from solanaceous hosts is being published elsewhere.—G. RANGASWAMI and C. N. SAMBANDAM, Department of Agriculture, Annamalai University, Annamalainagar, Madras State, India.

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A TRANSFERABLE PLASTIC CULTURE-TUBE LABEL

In 1939 Blaisdell [1] devised a permanent transferable culture-tube label. The tin-backed label with a cardboard or plastic writing surface is no longer manufactured and consequently is not easily obtainable. Since we are isolating and maintaining mycorrhizal fungi in culture it seemed most desirable to have a similar label that we could easily prepare in the laboratory. By experimenting with an inexpensive and readily obtainable plastic we devised a very suitable label.

Vinyl plastic sheets, .030 gauge with one side unpolished [2] were cut into strips 1″ × 2¼″ with a paper cutter. Three or four strips at a time were placed in water maintained just at or slightly below the boiling point. The plastic quickly became pliable. The strips were individually removed from the water with a pair of forceps. Each strip, polished side toward the glass, was molded around a prewarmed 16 mm culture tube with the thumbs and then held under cold running tap

[1] Blaisdell, Dorothy J. 1939. A permanent transferable culture-tube label. Phytopathology **29**(8): 761–762.

[2] Obtained from Transparent Products Corporation, P. O. Box 15924, Los Angeles 15, California.

water for a few seconds. The plastic hardened into a cylindrical shape (FIG. 1).

The plastic label is very resistant to changes in shape and it retains a springy quality. It will, of course, not withstand high temperatures. Re-use of the label will not affect its grip on a culture tube that has a diameter greater than the mold. Squeezing the sides of the cylinder together will not permanently reduce the size unless the plastic is first softened.

The unpolished outer surface offers a variety of possibilities for marking. Pencil is effective and is easily erased. Inks may be used and removed. We are using a permanent ink that is applied with a fine, felt-tipped pen. The ink is resistant to smearing and may be quickly removed by dipping the label in benzene for a few moments. Prolonged

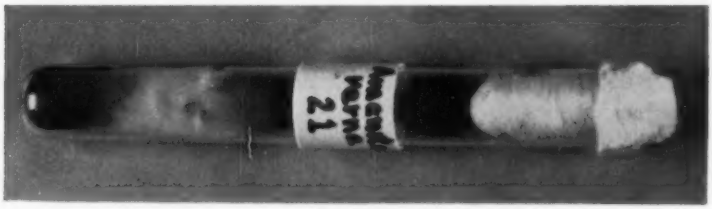

FIG. 1. A plastic transferable culture-tube label.

immersion in benzene will soften the plastic.—EDWARD HACSKAYLO, Plant Physiologist, Forest Physiology Laboratory, Forest Service, U. S. D. A., Beltsville, Maryland.

OLD TOMBSTONE INSCRIPTION RECORDING DEATH OF A FAMILY FROM MUSHROOM POISONING

In order that it may be available in "Mycologia" I am quoting here the inscription on a tombstone in the cemetery of historic Trinity Episcopal Church, Fishkill, Dutchess County, New York, that records the death of a father, mother and young son from mushroom poisoning. It reads:

"In memory of William Gould, who died on October 2d., 1838, aged 34 years, 5 months, 28 d's; also Sarah, his wife, who died October 2d, 1838, aged 35 years, 2 months, 2 d's; also Charles, their son, who died October 3d., 1838, aged 4 years, 4 days. Natives of the town of Noth. Wotton, England. Poisoned by eating Fungi. (toadstools)."

Poucher and Reynolds (**3**, p. 83) in their quotation of this inscription give the name of the town in England as "North Wootton." With a town by this name in each Dorset, Norfolk and Somerset counties, England (cf **1**, p. 740), it remains a question from which one this Gould family migrated to America. Their tombstone stands in the churchyard to the right of Trinity Church (see illustration by Ladd, **2**, opp. p. 42) and not far from the rear of the edifice.

My information on the lore of Trinity Church, including the presence in the churchyard of the tombstone in memory of the Gould family, emanates from my occasional visits to relatives at Fishkill. On such a visit, September 18, 1959, I again viewed the Gould tombstone. The following day, as circumstances developed, one of my relatives directed the rural correspondent of the "Poughkeepsie New Yorker" to the tomb. This led to his article in the Sunday issue of the paper (Oct. 4, 1959, editorial page) that begins with a quotation of the monument's inscription. "The warning is timely," he continues, "because this is just the time of year when amateurs go forth and pick mushrooms in our fields and pastures, just the time of year, in fact, when the Gould family went forth to pick them. And just the time of year when others might be mistaken, even as Mr. and Mrs. Gould."—ANNA E. JENKINS, National Fungus Collections, Plant Industry Station, Beltsville, Maryland.

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3. **Poucher, J. W., and H. W. Reynolds.** 1924. Old gravestones of Dutchess County, New York. Nineteen thousand inscriptions. Poughkeepsie, N. Y. 401 p.

MYRIANGIALES SELECTI EXSICCATI, FASCICLES 1–10 (NOS. 1–500)

For convenience of reference, our notices or accounts of our series "Myriangiales selecti exsiccati," fascicles 1–10, numbers 1–500 (1940–1957), are here cited:

Jenkins, A. E. and Bitancourt, A. A. 1942. "Myriangiales selecti exsiccati," fascicle 1. Mycologia **34**: 214–215.[1]

[1] Chronica Botanica (**7**: 192. 1942) recorded the issue of "Myriangiales selecti exsiccati;—Fasc. 1 . . . Edd. A. E. Jenkins and A. A. Bitancourt."

—— **and** ——. 1944. Myriangiales selecti exsiccati. Soc. Bras. Agron. Bol. **7**: 153–166. (Abstract in Rev. Appl. Mycol. **25**: 280. 1946.)
—— **and** ——. 1946 [1947]. Myriangiales selecti exsiccati, fascículos 2–6 (Números 51–300). Soc. Bras. Agron. Bol. **9**: 157–164. (Abstract in Rev. Appl. Mycol. **28**: 143. 1949.)
—— **and** ——. 1947 [1949]. Myriangiales selecti exsiccati, fascículi 7–8 (Númeri 301–400). Soc. Bras. Agron. Bol. **10**: 143–151.
—— **and** ——. 1953 [1954]. Myriangiales selecti exsiccati, fascicle 9 (Nos. 401–500), issued April 1953. Asa Gray Bull. (n.s.) **2**: 415–416.
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—— **and** ——. 1955. Myriangiales selecti exsiccati, fascículos 9 (Nos. 401–450) e 10 (No. 451–500). Soc. Bras. Agron. Rev. **11**: 7–12.
—— **and** ——. 1956. Myriangiales selecti exsiccati, fascículos 1–10, Números 1–500 (1940–1954), indices e outras listas pertinentes a essa coleção. Soc. Bras. Agron. Rev. **12**: 41–68.[2]

Our first article of 1953 and that of 1956 lists the institutions in which the 10 complete fascicles are deposited. The article of 1956 also lists the places of deposit of the 15 partial sets and sundry specimens.

In a special cover, folders of the labels of the 50 specimens issued in each of the 10 fascicles may be requested from the National Fungus Collections or the Instituto Biológico (Secção Fitopatológico).—ANNA E. JENKINS, National Fungus Collections, Plant Industry Station, Beltsville, Maryland, and A. A. BITANCOURT, Instituto Biológico, São Paulo, Brazil.

ELSINOË ON BOUCHEA PRISMATICA IN CUBA

For the new species of Elsinoë on *Bouchea prismatica* that I reported from Cuba in 1953,[1] with a description of it in English only, a diagnosis in Latin is herewith provided.[2]

Elsinoë boucheae sp. nov.

Laesiones in caulibus subrotundae vel elongatae, marginibus elevatis definitis, brunneis usque nigris cinctae, centro planae vel subdepressae, sordide albae, 0.5–4 × 0.4–2.0 mm; laesiones foliolares hypophyllae in venis, obscurae, elevatae, 1.0–3.0

[2] Corrections to this compilation (Washington, 4 p., 1957. Processed) are available in the National Fungus Collections and the Instituto Biológico (Secção Fitopatológico).

[1] Kevorkian, A. G. 1953. A spot anthracnose disease of *Bouchea* in Cuba. Phytopathology **43**: 406 (Abst.).

[2] For this rendering of the description in Latin I am indebted to Miss Edith K. Cash.

× 0.2–0.5 mm; ascomata subrotunda, per laesiones irregulariter dispersa, in strato exteriori prosenchymatis definiti hyalina 80 μ crassi evoluta, e pseudoparenchymate hyalino vel flavidulo efformata, epithecio stratibus 2–4 cellularum obscurarum applanatarum composito, 80–120 × 30–45 μ; asci globosi, 16–18 μ in diametrum; ascosporae usque ad 8, 3-septatae, hyalinae, 10–12 × 3–5 μ.

On stems and leaves including petioles of *Bouchea prismatica* (L.) Kuntz (Verbenaceae) Cienfuegos, Las Villas Prov., Cuba.

SPECIMEN EXAMINED: Roadside between Harvard House and Casa Catalina, Soledad, Cienfuegos, Cuba, October 30, 1941, *A. G. Kevorkian.* Deposited in National Fungus Collections, Plant Industry Station, Beltsville, Maryland (No. 90444), and Herb. Secção Fitopat., Inst. Biol., São Paulo, Brazil (No. 5165).—ARTHUR G. KEVORKIAN.

NEW SPECIES OF DISCOMYCETES FROM INDIA—I

Under the above-mentioned title will be published some new species of Discomycetes collected by me from the Indian subcontinent. These species have partly been described as "Taxonomic species" in my book entitled "A Preliminary Study of the Indian Discomycete Flora" (Batra, **1960**). More exhaustive work was needed and I therefore omitted the Latin diagnoses.

I have examined the type specimen of *Sphaerospora tuberculata* Thind, Cash & Sethi (**1958**) and am convinced that this is a species of *Trichophaea.* Since there is already a species *Trichophaea tuberculata* (Seaver) Kanouse (**1958**), my previously proposed new combination *T. tuberculata* (Thind, Cash & Sethi) Batra (**1960**) was an error. I therefore propose the new name **Trichophaea himalyaensis** nom. nov. for this fungus (Basionym: *Sphaerospora tuberculata* Thind, Cash & Sethi, Mycologia **49**: 831. **1958**). I have also examined the type specimen of *Humaria mussooriensis* Thind, Cash & Sethi (**1958**). This is another species of *Trichophaea* and hence a new combination, **T. mussooriensis** (Thind, Cash & Sethi) comb. nov. is proposed with *Humaria mussooriensis* Thind, Cash & Sethi, Mycologia **49**: 834. 1958, as the basionym.

Colors in quotation marks are taken from Ridgway (**1912**). The

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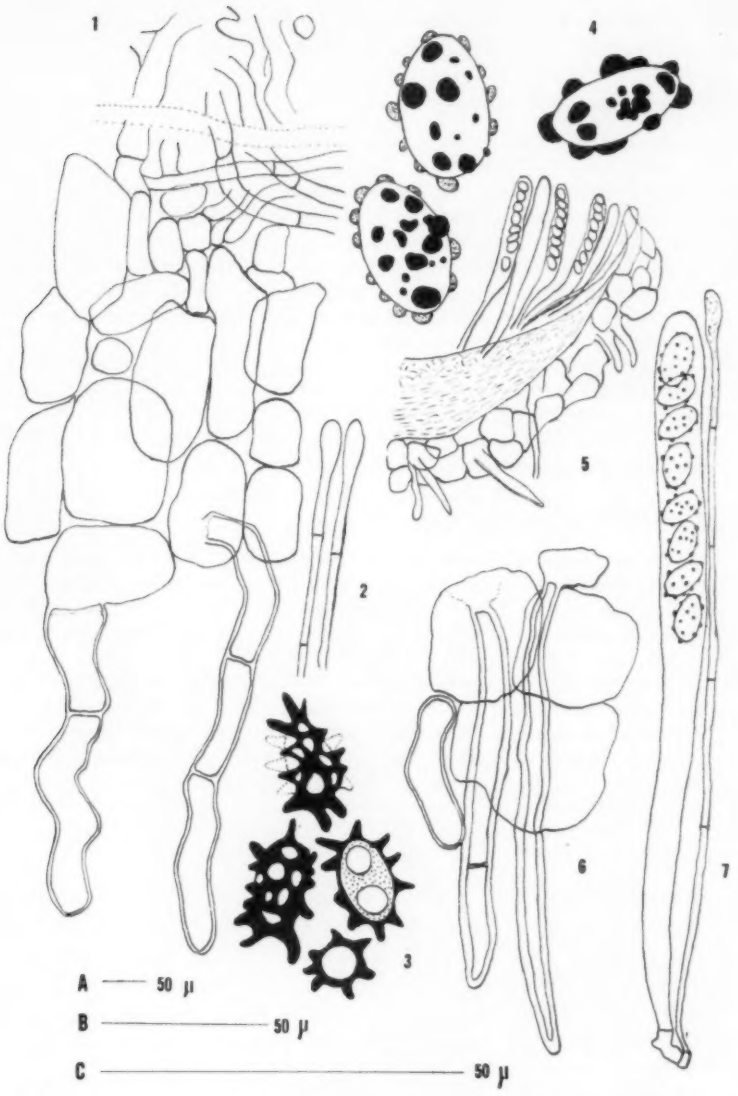
FIGS. 1–3. *Aleuria rubra.* 1. Part of a T. S. of apothecium showing ectal and part of medullary excipulum, × 500. 2. Upper portion of paraphyses, × 500. 3. Ascospores, × 1100. FIGS. 4–7. *Scutellinia uliginosa.* 4. Ascospores, × 1100.



5. T. S. of apothecium showing hymenium, subhymenium, medullary excipulum and ectal excipulum, × 130. 6. Part of ectal excipulum at higher magnification, × 500. 7. An ascus and a paraphysis, × 500. Scale A for FIG. 5; scale B for FIGS. 1, 2, 6, 7; scale C for FIGS. 3 and 4. All figures are drawn with the aid of camera lucida.

type specimens have been deposited in the Department of Plant Pathology Herbarium (CUP–I), Cornell University, Ithaca, New York.

Aleuria rubra Batra, sp. nov. (FIGS. 1–3)

Apothecia gregaria vel sparsa, sessilia, juncta firmiter terrae; discus cupulatus vel discoideus, fragilis, "Vivid Red," 2–6 cm diam., pilosus; pilis penitis non defixis; pilis exteris brevibus, densis, fuscis, septatis, deciduis, 200–300 × 9–11 μ; apothecii secti hymenium 256–300 μ, aurantiacum; subhymenium e compacta textura intricata, minus aurantiacum, 60–70 μ; excipulum medullare raro textura intricata, 310–330 μ; excipulum exterius textura prismatica, 160–180 μ, cellulae subhyalinae, magnae duobus usque quinque stratis, intermixtis inter cellulas hyphas factas in pila non ramosa; asci operculati, cylindracei, octosporidei, ad basim attenuati, jodo non coerulescentes, (250–)300–330 × 10–12 μ; ascosporae ellipsoideae, apiculatae, subhyalinae, biguttulatae, parietibus asperis, episporio reticulato, (18–)24–26(–28) × 10–13 μ reticulis inclusis; paraphysibus linearibus, clavatis, septatis, non furcatis vel furcatis, aurantiacis, apice crassatis, 310–340 × 2–3 μ, apice ad 9 μ in diam.

Holotypus CUP–I 6; super terram nudam; loco typi Mussoorie, alt. 7000 ft., Indiae Orientalis; etymologice ex Latino, rubra = RED.

Scutellinia uliginosa Batra, sp. nov. (FIGS. 4–7)

Apothecia gregaria vel sparsa, sessilia; discus cupulatus vel discoideus, fragilis, aurantiacus, pilosus, 2–8 (10) mm diam.; pilis penitis defixis densis, fulvis, parce septatis vel non septatis, crassis parietibus, tenacibus, 150–200 × 10–12 μ; pilis exteris brevibus et parcioribus, pilis penitis defixis; apothecii secti hymenium aurantiacum, 250–300 μ; subhymenium et excipulum medullare textura intricata, minus aurantiacum, 180–200 μ; excipulum exterius textura angulari, 68–100 μ; asci operculati, cylindracei, octosporidei, ad basim attenuati, jodo non coerulescentes, 220–270 × 12–15 μ; ascosporae ellipsoideae, subhyalinae, parietibus asperis, papillis 1–2 μ crassis, uniguttulatae, 20–25 × 11–12 μ; paraphysibus non ramosis, clavatis, septatis, aurantiacis, apice crassatis, 250–300 × 2–3 μ, apice ad 10 μ.

Typus CUP–I 32; super terra uliginosa; loco typi Rajpura (U. P.), alt. 4000 ft., Indiae Orientalis; etymologice ex Latino, uliginosus = *swampy* (*ground*) ex habitationi.

I am indebted to my former colleague, Dr. William C. Denison of Swarthmore College, where much of this work was conducted, for help during progress of this study. This research has been supported by grant no. G-9041 from the National Science Foundation to Dr. Richard P. Korf, Cornell University, "Discomycete Flora of Asia."—LEKH R. BATRA, Department of Botany, University of Kansas, Lawrence, Kansas.

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EFFECTS OF ULTRA-LOW TEMPERATURES ON THE VIABILITY OF SELECTED FUNGUS STRAINS

To insure viability of stock cultures in fungal collections, various methods of preservation have been developed. Covering of slants with sterile mineral oil (**2**) and dispersal of spores in soil (**1**) have been used. Deep freezing of fungal cultures (**3**, **6**) has been reported practicable. The effects produced by the processes upon the preserved cultures are not, however, experimentally controlled. The prospects of success or failure are therefore uncertain. The viability of cultures preserved by any of these methods can hardly be insured for more than five years.

The freeze-drying process (**4**) has been found applicable to the preservation of the life of many microorganisms and biological specimens. But unfortunately according to our experience great numbers of fungi do not survive when treated by the freeze-drying process.

This paper reports a successful treatment of fungus cultures at ultra-low temperatures which is proposed as an alternative to the conventional freeze drying (lyophilization) for maintaining stock cultures.

The organisms chosen for this study did not survive lyophilization as now practiced. The fungi were *Rhizoctonia solani* (#10184 and #10186)[1] and *R. microsclerotia* (#10187) (Mycelia Sterilia); *Botrytis paeoniae* (#12482) (Fungi Imperfecti); *Pythium aphanidermatum* (#12192), *P. debaryanum* (#9998 and #10393), *Phytophthora infestans* (#9901), *P. cinnamomi* (#11928), *P. palmivora* (#13708), *Syzygites megalocarpus* (#11807) and *Choanephora cucurbitarum* (#12997) (Phycomycetes).

The slow freezing apparatus and the general procedure for freezing were those described by Hauschka et al. (**5**) with minor modifications.

One ml of 10% glycerol in demineralized water was introduced aseptically into a sterile 10 × 75 mm Pyrex test tube by means of a 1 ml pipette. With a sterile needle a good portion of test material (mycelium and spores or mycelium alone) from a well-grown test tube culture was transferred into the above medium in the numbered vial. The vial was sealed off with a "Bernz-O-Matic" propane torch. Four sealed vials

[1] American Type Culture Collection accession numbers.

containing cultures and an unsealed control vial containing medium only were clipped onto 5 metal canes and lowered into an ethyl alcohol bath in a polyethylene beaker for precooling to 7° C.

After precooling for an hour, the polyethylene beaker with the test samples and the control was placed in the freezing bath as described by Hauschka et al. (**5**) and the slow freezing was begun.

The temperature drop from 0° to −25° C was measured against time by inserting a thermometer into the accompanying control vial.

The slow freezing process was stopped when the temperature reached −25° C. The vials were transferred immediately to a portable dry-ice-ethylene glycol bath (temperature −79°) for subsequent transfer of the frozen material into a model LNR-25-B liquid nitrogen refrigerator (Linde Co., Tonawanda, New York). In the present set-up with four vials of material being frozen simultaneously, the temperature drop of the test material from 0° to −25° required 45 minutes. In 15 tests an average drop in temperature of 1.7° per minute occurred.

The model LNR-25-B nitrogen refrigerator maintains a constant temperature of −195.5° which is below the liquid nitrogen level but −184.5° above the liquid level. The frozen vials were left in this liquid nitrogen refrigerator for 3 days; then they were put immediately into dry-ice-ethylene glycol slush and thence plunged into a 37° constant-temperature water bath and swirled until thawed. It took less than a minute to complete the thawing.

The vials were then opened and the cultures tested for viability, and the solid contents were transferred with a sterile needle into test tubes containing appropriate media for their growth. The liquid contents remaining in the vials were also transferred with a pipette to other test tubes containing the same media in order to include detached spores or mycelial fragments. After the period of incubation normal for each strain, viability was determined by observation of visible growth in the culture tubes.

Growth resulted in all the subcultures that were obtained by transfers from the frozen vials in the usual incubation period for each strain. No detectable changes in the cultural characteristics of any of the 12 strains tested were noted.

Syzygites megalocarpus, strain #11807, was cultured, frozen and thawed five times in succession. Its cultural characteristics remained unchanged.

The temperature zone where injury to cells results from freezing was suggested by Meryman (**7**) to be somewhere between −5° and −10°. If the specimen survives freezing in this range, further decrease

in temperature would theoretically cause no further change in the degree of dehydration. In our experiments the slow freezing process was extended to −25° so that the organisms involved would be frozen safely beyond the danger zone.

As the temperature passes below −100° (the glassy transformation temperature), chemical reactions are presumably completely arrested. Because the metabolic activities of living organisms including genetic changes are composed of chemical reactions, under the low temperature storage described, theoretically the genetic changes are arrested and life is preserved indefinitely. Verification of such an hypothesis can be realized only after long term storage and subsequent testing of viability and other characteristics.

Much work has been done by workers in other fields of biology (**8**, **9**) to demonstrate that living specimens of various types of organisms and biological materials are actually preserved successfully at low temperatures. These preliminary studies confirm the previous findings and add fungi to the list of organisms that can be successfully preserved in this way.

We are grateful to Doctor C. W. Hesseltine of the Northern Utilization Research and Development Division of the U. S. Department of Agriculture who kindly checked the cultural characteristics of *Syzygites megalocarpus* after the tests had been run.—SHUH WEI HWANG, American Type Culture Collection, 2112 M Street, N.W., Washington 7, D. C.

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REVIEWS

AINSWORTH & BISBY'S DICTIONARY OF THE FUNGI, by G. C. Ainsworth. 5th ed. 547 p. (including 14 p. of line drawings). Commonwealth Mycological Institute, Kew, Surrey. 1961. Price, 30s.

Mycologists again are indebted to Dr. Ainsworth and the late Dr. Bisby for another edition of this invaluable work. More than five thousand corrections or alterations have been made to the text of the Fourth Edition (1954). Much new material, including some 500 more generic names, have been included. Dr. G. W. Martin has revised his outstanding "Key to the families of fungi." Those who use the Dictionary soon become aware of the great amount of effort that has gone into the preparation of the work. Its completeness and correctness are remarkable.

Everyone interested in the fungi should have a copy of the work at hand.—CLARK T. ROGERSON.

HERB. I.M.I. HANDBOOK, Methods in use at the Commonwealth Mycological Institute. 103 p., 6 pls., 8 figs. Commonwealth Mycological Institute, Kew, Surrey, England. 1960. Price, 12s. 6d.

Here is a "guide" that should be on the desks of mycologists and plant pathologists. It is a summary of the activities of the C.M.I. and provides invaluable information on the methods of handling fungi found to be most successful after many years of trial by the C.M.I. staff. The book has been dedicated to E. W. Mason as a token of appreciation of his life's work of helping to bring order in the classification of fungi, particularly the microfungi associated with disease of plants. Included are sections on the history of C.M.I., by S. P. Wiltshire; the herbarium, by M. B. Ellis; how to describe a fungus, by F. C. Deighton; laboratory methods used in the culture collection, by H. A. Dade; the treatment of important pathogenic phycomycetes, by Grace M. Waterhouse; bacteria and the herbarium, by A. C. Hayward; collecting fungi, by C. Booth; collecting in the tropics, by F. C. Deighton; and collecting in the clinic, by G. C. Ainsworth. Information on C.M.I. publications is given.

Curators, herbarium and laboratory mycologists, and plant pathologists working with fungi should be stimulated by the many ideas which fill this book.—CLARK T. ROGERSON.

MUSHROOMS OF THE GREAT SMOKIES, by L. R. Hesler. xii + 289 p., *illus.* University of Tennessee Press, Knoxville, 1960. $5.50.

Amateur naturalists must revel in the growing number of books being written primarily for them by specialists in various areas of natural science. Prominent among these books are the field guides, which provide a great deal of information for a reasonable outlay of cash. These guides are of such size that they may be handled easily on outdoor excursions, for their aim is to answer the question, "What is it?" when the object—be it animal, plant, or mineral—toward which the question is directed is in hand, or at least in sight. The information the guides provide to answer the question is in the form of illustrations and descriptions of readily observable characteristics.

I have generally considered 1934 as the beginning of the field guide era in this country, that being the year in which Peterson's *A Field Guide to the Birds* appeared. Therefore, I was recently surprised to come across a *Field Book of Common Gilled Mushrooms* by Thomas, published in 1928. Several guides for the higher fungi have appeared since that time, and now Hesler has drawn on his nearly forty years of experience in the Great Smoky Mountains National Park to produce "a field guide to some mushrooms and their relatives." The book's utility is not limited to the Park, however, for many of the species included occur also far beyond the Park's borders.

Hesler's book, attractively designed by Charles Counts, has a striking cover with a color photograph of *Amanita caesarea* and impressed printing in silver, all on a black background. The text itself is organized under three headings.

Part One, "Mushrooms in Nature," deals briefly with a diversity of topics. The habitats offered by the Park are considered with emphasis on their relation to the rich fungous flora. Approximately 2000 species have been found in the Park. Nine hundred of these are mushrooms, a term restricted to the gill fungi, but Hesler estimates that the eventual number of mushroom species will be near 1500. The development and gross morphology of a mushroom are explained, and attention is rightly called to the importance of microscopic study in making accurate determinations of many species.

Mycophagists receive appropriate advice. The descriptions of poisoning by five species, especially that by *Amanita brunnescens*, are sufficiently gruesome to underscore the necessity for caution. A bow to the spectacular is inappropriately made with a section on hallucinogenic mushrooms. The section has two sentences on *Amanita muscaria*, found

in the Park, and two paragraphs on the psilocybes, found in the Huautla region of Mexico.

Part One concludes with sections on fairy rings, mycorrhizal associations, autodigestion, and luminescence.

Parts Two, "Mushrooms in the Park," and Three, "Mushroom Relatives" (i.e., rusts, polypores, boletes, gasteromycetes, discomycetes, etc.) constitute the major portion of the book. Each begins with a yoked key to genera or groups of genera and directions for its use. These keys serve well enough for the species included in the book, but offer pitfalls to those who attempt to determine the genus of several excluded species which can be thought of offhand. To expect otherwise in a book of this sort is unjust, however, in view of the characteristics now emphasized in delimiting genera of higher fungi. The inclusion of appropriate page numbers after the names in the keys throughout the book would have been an aid to those using it.

Each genus or group of genera is discussed and usually provided with a key to included species. The species, 183 in all, are described briefly and illustrated with first-rate black and white photographs, most of which were taken by Hesler himself. Common names and indications of edibility, or lack of it, are given. An excellent feature is the inclusion in the habitat information of the time of year when and the Park areas where the species may be found. The front end paper of the book has a Park map which locates the various areas.

The book ends with a short glossary, a suitable bibliography, and a good index. The glossary defines two words as parts of speech which they are not and gives a definition of "species" which surely was not intended.

Although the delightfulness of *Mushrooms of the Great Smokies* is lessened by a writing style which seems stilted in places and which is marred by occasional grammatical and punctuational errors, the book has many things to recommend it to anyone interested in the higher fungi. Its main attraction for professional mycologists is the excellent photographs, some of species for which such have not been previously published.—ROBERT L. SHAFFER.

SABOURAUDIA, Journal of the International Society for Human and Animal Mycology. E. & S. Livingstone, Limited, 15–17 Teviot Place, Edinburgh 1, Scotland. Subscription price, $12.00 per volume, $3.00 per issue.

It is a pleasure to announce the birth of another journal devoted to

mycology. The first issue of volume one was released in January, 1961 (72 pages). It includes a memorial to the "father" of medical mycology, Raimond Sabouraud, in whose honor the journal is named. Also included is a statement about The International Society for Human and Animal Mycology by its President, Juan E. Mackinnon. Members of this Society (ISHAM) receive the journal upon payment of the annual dues which are now set at $3.00 per year. It is anticipated that annual dues will be increased as the journal increases in volume size.

Eight technical articles (Yeasts from Human Sources; Pathogenicity of *Candida albicans* and *Candida tropicalis;* Moniliasis in Partridges; Studies of the Invasive, Mycelial form of *Candida albicans;* Reproduction and Pathogenicity of *Cryptococcus neoformans; Nannizzia incurvata* gen. nov., sp. nov., a perfect state of *Microsporum gypseum;* The Perfect States of *Keratinomyces ajelloi, Trichophyton terrestre,* and *Microsporum nanum;* and The Extra-human Occurrence of *Trichophyton tonsurans* var. *sulphureum* in a Residential School) form the bulk of the issue. Obituary notices, reviews, and Society information conclude the issue.

The format of the journal is excellent; good paper is used; many black-and-white photographs complement the articles.

The editorial board truly indicates the international scope of the journal. J. C. Gentles (Great Britain) is editor; W. Kaplan (U.S.A.), P. N. Kaschkin (U.S.S.R.), C. da Silva Lacaz (Brazil), F. Mariat (France), A. González Ochoa (Mexico), H. P. R. Seeliger (Germany), T. Tsuchiya (Japan), and G. A. de Vries (Netherlands) are members of the board.—CLARK T. ROGERSON.

A STUDY OF RUSSULA TYPES, by L. R. Hesler. Memoirs Torrey Botanical Club 21 (2): 1–59. 318 figs. Price, $1.50.

This paper presents Dr. Hesler's studies of type or authentic specimens of 159 North American species of *Russula.* Special attention has been given to the structure of the pileus cuticle and to the ornamentation of the basidiospores. For each species excellent drawings of basidiospores and elements of the epicutus are included. The species are arranged alphabetically with citations to the original description, and with descriptions of basidiospores, epicutis, and, for some species, cheilocystidia and pleurocystidia.—CLARK T. ROGERSON.

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